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THE MODE OF INHERITANCE OF RESISTANCE TO PUCCINIA GRAMINIS WITH RELATION TO SEED COLOR IN CROSSES BETWEEN VARIETIES OF DURUM WHEAT [1]

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INTRODUCTION

It has long been recognized that the production of economically desirable varieties of wheat resistant to *Puccinia graminis tritici* Erikss. & Henn. would be of great value. Until recently, hybridization experiments with this end in view were based upon a very incomplete knowledge of both the exact nature of the pathogene and the nature of the inheritance of resistance, and consequently, satisfactory results were not obtained. A knowledge of the mode of inheritance of certain desired economic characters of wheat together with the discovery that there are several biologic forms of stem rust of wheat has been of much importance in placing breeding studies on a definite basis. Several varieties belonging to different species or subspecies of wheat have been found to be resistant to certain biologic forms. It is hoped that by hybridization, resistance to all biologic forms may be combined in one variety. The present problem was attacked chiefly for the purpose of determining whether the resistance of two varieties, having different factors for resistance, may be combined in the progeny of a cross between them.

REVIEW OF PREVIOUS INVESTIGATIONS

Since 1894, when Eriksson (*7*) [3] discovered biologic specialization in the black stem rust, numerous investigators (*6*, *9*, *25*) have corroborated his work. The reports of Ward, Evans, and others (*32*, *8*, *9*) indicated that biologic forms might change rather rapidly as a result of host influence but more recent work by Stakman and others (*27*, *30*) showed that biologic forms were apparently quite stable. Previous to 1916, it was generally believed that only one form of black rust attacked wheat.

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[3] Reference is made by number (italic) to "Literature cited," p. 995-996.

However, the investigations of Stakman and others (*28*, *29*, *21*, *20*, *26*) have proved that there are a number of biologic forms of this rust.

A total of 37 different biologic forms of *Puccinia graminis tritici* have been isolated at the Minnesota station by Stakman and Levine (*25a*). No one form has been found to attack all varieties of Triticum species and no wheat variety has been found to be resistant to all rust forms, except Khapli emmer, a variety of *T. dicoccum*. The exact distribution of each biologic form is not known (*19*). Perhaps not half of the 37 forms are present in any one wheat-growing district. For example, only 14 forms had been collected up to December, 1921, in western Canada (*23*). However, as it is possible that some forms may spread into new districts, the problem of breeding for resistance means working for a variety resistant to all known forms of rust.

The study of disease resistance in wheat dates back over a century. According to Biffen (*3*), Thomas Knight, in 1815, suggested that disease-resistant varieties might be raised, and Farrar, in 1889, stated that susceptibility to rust was hereditary in wheat. Biffen (*3*), in 1905, crossed Red King, a variety susceptible to *Puccinia glumarum* (Schm.) Erikss. & Henn., with Burt, a resistant variety. The F_1 progeny was susceptible and the F_2 progeny segregated into susceptible and resistant in a 3: 1 ratio. Later, Biffen (*4*) stated that the relatively immune hybrid forms of wheat bred true and that the immunity was independent of any discernible morphological character, that is, the factors for resistance were inherited independently of other factors. More recently, Biffen (*5*) reported the production of a rust-resistant wheat which likewise possessed other desirable economic characters.

Some excellent results in breeding for disease resistance in crop plants have been reported by various workers, but the problem is rarely as difficult as in the case of stem rust of wheat. Gaines (*12*) crossed two varieties of wheat highly resistant to bunt and obtained in the F_2 some plants which were more susceptible than either parent and a large percentage which were immune. He concluded that the two varieties had different factors for resistance. Garber (*13*), in 1921, found that resistance to *P. graminis avenae* Erikss. & Henn. was a dominant character, a ratio of 3 resistant to 1 susceptible being obtained in F_2.

A definite grouping of the wheat subspecies according to their reaction to *P. triticina* Erikss. was made by Vavilov (*31*) in 1914. The use of interspecific crosses in breeding for rust resistance is liable to some complication owing to the linkage of certain factors. In 1917, Freeman (*10*) reported cases of linked quantitative characters in wheat crosses. In 1919, Hayes, Parker, and Kurtzweil (*16*) found that in crosses of varieties of *T. vulgare* with varieties of *T. dicoccum*, resistance was dominant. In crosses of *T. vulgare* with *T. durum* susceptibility was dominant and in F_2 there was strong linkage between rust resistance and the durum character. The significant fact, however, was that some crossing-over occurred. It was apparent, therefore, that there was a possibility of transferring the resistance of durums or emmers to common wheats.

Puttick (*24*), in 1920–21, studied the reaction of the F_2 generation of a cross between a common and a durum wheat to two biologic forms of *P. graminis tritici* to which these varieties reacted reciprocally. F_2 seedlings were inoculated with one rust and later the infected leaves were cut off and the plants were inoculated with a second rust form. All gradations between complete susceptibility and immunity to both

forms of rust were obtained. Some of the progeny appeared to be resistant to both forms.

More recently Melchers and Parker (*22*) found that a single factor was responsible for the resistance in crosses of Kanred with Marquis, Preston, and Haynes Bluestem, which were inoculated with a single form of rust. Segregation in F_2 was in a 3:1 ratio of resistant and susceptible plants. The results were verified by carrying the study through the third generation.

Aamodt (*1*, *2*), in 1921, studied the inheritance of resistance to several biologic forms of *P. graminis tritici* in a cross between Kanred, a winter wheat, and Marquis, a spring-sown variety. Kanred is resistant to a number of biologic forms whereas Marquis is susceptible to most of them. F_3 seedling plants inoculated with a form of rust to which Kanred was immune and Marquis susceptible proved either resistant or susceptible. Families, homozygous for spring habit of growth and for resistance to all the forms of rust to which Kanred was resistant, were obtained in the F_3. Here a single factor apparently determined the reaction to several biologic forms, to 11 of which Kanred is immune.

Of the 37 known biologic forms of stem rust 21 were found in the area where hard red spring wheats are grown.[4] The most recent study in disease-resistance breeding at the Minnesota station shows the possibility of synthetically producing a desirable *vulgare* variety of wheat that will be resistant to all of these biologic forms (*17*). This is to be accomplished by means of several crosses and double crosses between varieties having the desired resistance and those having the necessary bread-wheat qualities for yield, milling, baking, etc.

In the present investigation use was made of a cross between two varieties of durum wheat which react reciprocally to two biologic forms of rust, each being resistant to the form to which the other is susceptible. It was hoped that some F_3 families would prove resistant to both forms of rust. In addition to a study of the genetic factors concerned with resistance the relation of seed color to rust resistance was also considered. That resistance to several biologic forms is governed by a single factor, in the Kanred × Marquis cross, indicates the importance of learning as much as possible about the inheritance of resistance to various rust forms in different crosses.

MATERIALS AND METHODS

The F_3 families[5] of two *T. durum* crosses, Kubanka 8 (C. I. 4063) × Pentad (C. I. 3322) and Mindum (Minn. No. 470) × Pentad, were used. Kubanka No. 8 is a white-seeded selection from common Kubanka (C. I. 1440). Pentad is a red-seeded variety selected at the North Dakota station. Mindum is a white-seeded selection of the Arnautka type. Seedling plants from each F_3 family were inoculated in the greenhouse with two biologic forms of *P. graminis tritici*. The crosses were made in 1920, and the F_1 generation was grown in the greenhouse at Washington, D. C., during the winter of 1920–21. The F_2 generation was grown in the plant-breeding nursery at University Farm, St. Paul, Minn., during the 1921 season, the plants being harvested individually.

[4] Unpublished results of Stakman and Levine.

[5] The term "F_3 family" is used in this article for the seedling plants grown from the seeds of a single F_2 plant.

The material was a part of that which was being used for the purpose of obtaining resistant wheat for growing in the Northwest. This problem was being cooperatively investigated by the Sections of Plant Breeding and Plant Pathology of the Minnesota Agricultural Experiment Station, and the Office of Cereal Investigations of the United States Department of Agriculture. The individually harvested F_2 plants were furnished to the present writers. The biologic forms of rust were some of those which were being studied by M. N. Levine.

Plants of each F_3 family were grown in 4-inch pots in the greenhouse, one family in each pot. All pots of soil were steam sterilized immediately before use and the seed was sown at a uniform depth. A large proportion of the hybrid seed as well as of the parental seed was infected with Helminthosporium and consequently the number of plants per pot varied between 8 and 20. The seedlings were grown in a seedling section of the greenhouse in which no rust-infected plants were kept. When they were about 3 inches high they were inoculated with fresh urediniospores of one of the two forms used. Only one rust form was worked with at one time. The pots were then placed in an incubation chamber for 40 to 48 hours with a number of control pots of the parental varieties inoculated in the same way. The two forms of rust were kept on well-separated benches to avoid accidental contamination of one with the other. An attempt was made to keep the temperature, moisture, and light condition the same for all plants. The methods used were essentially the same as those described by Stakman and Piemeisel (*29*).

Notes on the character of infection with both forms were taken from 15 to 19 days after the date of inoculation. The amount and rapidity of rust development was found to vary in response to environmental conditions. However, the character of infection was found fairly constant within certain limits, regardless of external influences.

The F_3 seedling plants were inoculated in sets of about 25 to 40 pots each. The total interval during which results were being recorded for the reaction to any one biologic form was approximately two weeks. In addition to running control plants of the parental varieties with each set of hybrid plants, one or more complete sets of "differential" varieties were inoculated during work with each form. In this way a complete control was obtained on the identity of the form being used. The differential varieties used were as follows: Little Club (C. I. 4066); Marquis; Kanred; Kota (C. I. 5878); Arnautka (C. I. 4072); Kubanka (C. I. 2094); Mindum (Minn. No. 470); Acme; Einkorn (C. I. 2433); Vernal emmer (Minn. No. 1165); and Khapli emmer (C. I. 4013).

The percentage of infected plants obtained, on the average, was very satisfactory. Of a total of more than 10,321 plants which were inoculated 796 showed no infection.

In recording the types of infection the symbols prepared by Stakman and Levine (*25a*) in their work on biologic specialization were used. These classes of host reaction and of corresponding types of infection resulting from inoculation of seedling wheat plants with spores of *Puccinia graminis tritici* are as follows:

Classes of host reaction	Types of infection [6]
Immune	0. No uredinia developed but definite hypersensitive areas present.
Very resistant	1. Uredinia minute and isolated; surrounded by sharp, continuous, hypersensitive areas; hypersensitive areas lacking uredinia also may be present.
Moderately resistant	2. Uredinia isolated and small to medium in size; hypersensitiveness present in the form of necrotic areas or circles; uredinia often surrounded by green islands.
Moderately susceptible (apparently resistant in the field)	3. Uredinia midsize; coalescence infrequent; development of rust somewhat subnormal; true hypersensitiveness absent; chlorotic areas, however, may be present.
Very susceptible	4. Uredinia large or varying from midsized to large, numerous and confluent; true hypersensitiveness entirely absent; chlorosis seldom present.
Resistant to moderately susceptible (apparently resistant in the field)[7]	X − Uredinia very variable; apparently includes two or more of the 0, 1, 2, and 3 types of infection on the same leaf blade; no mechanical separation possible; on reinoculation one type of uredinia may produce another; infection ill-defined.
Moderately susceptible to very susceptible (probably susceptible in the field)	X + Uredinia very variable; apparently including combinations of one or more of the types 0, 1, and 2, with type 4 on the same leaf blade; no mechanical separation is possible; on reinoculation one type of uredinia may produce a different type; infection ill-defined.

SELECTION OF BIOLOGIC FORMS

In October, 1921, about 15 of the 37 known biologic forms were available. The reaction of Mindum to these forms was known but Kubanka No. 8 and Pentad had not been tested. It was hoped that among the 15 forms some could be found that would react reciprocally on the parents of the crosses to be tested. Extensive studies disclosed that Forms I and XXXIV reacted reciprocally on Mindum and Pentad. However, similar success was not achieved with respect to Kubanka No. 8 and Pentad. Therefore, only one form, No. XXXIV, was used for the Kubanka 8 × Pentad cross.

The first rust form, designated as Form I, was collected at St. Paul, Minn., in September, 1915. When used for the present study it had been cultured through approximately 90 urediniospore generations and there was no indication of a change in its parasitic capabilities. Mindum is decidedly resistant to this form, whereas Pentad and Kubanka No. 8 are normally susceptible.

The second form, known as Form XXXIV, was collected by G. F. Puttick at Potchefstroom, South Africa, in April, 1921. When it was received at the Minnesota station it was evidently mixed with one other form. The separation of these forms was not completed until December, 1921. Since then more than seven generations have been produced without any indication of a change in the constancy of the form. In contrast to its reactions to Form I, Mindum is quite susceptible to Form XXXIV and Pentad is resistant. Kubanka No. 8 is susceptible to this form.

[6] These descriptions of types of infection are based on those given by Stakman and Levine (*25a*).
[7] Unpublished results of cooperative rust breeding work carried on at the Minnesota station.

ACCURACY OF THE RESULTS

In estimating the accuracy of the types of infection that appeared on the hybrids the behavior of the rust on pure varieties was taken as a criterion of the average amount of variation that occurred. In Table I the results of inoculations made with Form I on February 10 and 20 are given for a number of varieties, including Mindum and Pentad. In several cases, as those of Little Club and of Khapli, the results from the two periods are practically identical. In the majority of cases, however, the rust development from the inoculations of February 10 was weaker than from those inoculations made February 20. For example, einkorn had type 3 infections from the first inoculation but type 4 from the later inoculation. Similarly, with Kota, the February 10 inoculations produced type 3 infections whereas type 4 resulted from the February 20 inoculations.

TABLE I.—*A comparison of the types of infection produced by biologic Form 1 on several varieties of wheat at two different periods*

Group and variety.	Date inoculated.	Rust development.	Number of plants apparently not infected.	Types of infection.							Total of infected plants.
				0	1	2	X−	3	X+	4	
Common wheats:		*Days*									
Little Club	Feb. 10	17	.....	.....	.....	.....	.....	.....	.....	24	24
	20	16	.....	.....	.....	.....	.....	.....	.....	13	13
Marquis	10	17	.....	.....	.....	.....	.....	.....	.....	12	12
	20	16	2	.....	.....	.....	.....	.....	.....	12	14
Kanred	10	17	14	.....	.....	.....	.....	.....	.....	.....	14
	20	16	10	.....	.....	.....	.....	.....	.....	1	11
Kota	10	17	.....	.....	.....	.....	.....	14	.....	.....	14
	20	16	.....	.....	.....	.....	.....	.....	.....	15	15
Durum wheats:											
Arnautka, C. I. 1493.	10	17	.....	11	.....	.....	1	.....	.....	3	15
	20	16	.....	9	.....	.....	.....	.....	.....	4	13
Kubanka, C. I. 4063.	10	17	5	.....	.....	.....	.....	6	.....	2	13
	20	16	3	.....	.....	.....	.....	.....	.....	12	15
Acme	10	17	2	.....	.....	.....	.....	11	.....	2	15
	20	16	.....	.....	.....	.....	.....	.....	.....	11	11
Mindum	10	17	1	19	8	.....	.....	.....	.....	.....	28
	20	16	.....	30	.....	.....	.....	.....	.....	1	31
Pentad	10	17	4	4	3	.....	10	1	2	.....	24
	20	16	.....	.....	1	.....	3	.....	8	12	24
Einkorn	10	17	.....	.....	.....	.....	.....	14	.....	.....	14
	20	16	.....	.....	.....	.....	.....	.....	.....	14	14
Emmers	10	17	1	12	.....	.....	.....	.....	.....	.....	13
Vernal	20	16	3	11	.....	.....	.....	.....	.....	.....	14
Khapli	10	17	.....	.....	13	.....	.....	.....	.....	.....	13
	20	16	.....	.....	14	.....	.....	.....	.....	.....	14

The appearance of a type 4 infection on 1 out of 11 Kanred plants inoculated February 20 and similarly on 1 out of 31 Mindum plants, indicated accidental infection from some other form of rust, or impurity in the seed due to mechanical mixture or to natural crossing. The

Arnautka seed was known to be impure. This explains why 8 out of the 28 plants inoculated did not appear to be immune. Hayes (*14*) and Hayes and Garber (*15, p. 35–36, 77–78*) have reported from 2 to 3 per cent of natural crossing in wheat grown at St. Paul, Minn. A rather large difference is shown between the inoculations of February 10 and February 20 on Pentad. This demonstrates the value of running several pots[8] of the parental varieties with each set of hybrid families.

From the foregoing discussion it should not be concluded that the variations were so great as to obscure the results. The reaction of the F_3 plants to the different forms of rust was constant within certain limits and the fact that the plants which were tested were of the third hybrid generation, and consisted of families each arising from a single F_2 plant, makes the interpretation of the various reactions a fairly definite matter.

BASIS FOR INTERPRETING RESULTS

As the determination of the genetic nature of resistance was the chief object of this investigation, the results obtained were interpreted on the basis of the reactions of the parental varieties to the forms of rust used. The F_3 families were placed in five classes with reference to reaction to the different biologic forms, viz, resistant, near-resistant, heterozygous, near-susceptible, and susceptible. In the work with Form I the class designations "immune" and "near-immune" were used instead of "resistant" and "near-resistant" owing to the nature of the Mindum reaction.

F_3 families showing resistance similar to that of the resistant parent were termed resistant. Similarly, the F_3 families showing susceptibility equivalent to that of the susceptible parent were classed as susceptible. Those F_3 families having 9 or more apparently resistant plants and one plant showing susceptibility were classed as near-resistant. Likewise F_3 families with 9 or more plants showing susceptibility and one plant appearing resistant were placed as near-susceptible.

It is probable that some of the F_3 families placed in the near-resistant or near-susceptible class were in reality heterozygous. It is also probable that others placed in these classes were resistant or susceptible, respectively. Owing to the likelihood of the occurrence of certain errors, which have been discussed in a previous section of this paper, it was not possible to determine the exact genetic nature of the "near-resistant" and "near-susceptible" F_3 families. It was best, therefore, to leave these doubtful F_3 families in classes which by their names indicate their character.

All F_3 families not falling in one of the preceding four classes were termed heterozygous. The heterozygosity of various F_3 families varied from a preponderance of apparently susceptible plants to a preponderance of plants showing resistance. Of the five different classes the resistant class is of greatest importance, as it represents the F_3 families which appeared homozygous for resistance.

[8] The word "pot" is used throughout this article to denote the group of plants of a pure line parent variety growing in a single pot, such a group being comparable to a group of F_3 plants (an F_3 family) likewise growing in one pot.

EXPERIMENTAL RESULTS

THE SEED COLOR RATIO

The inheritance of seed color in wheat has been studied for a number of years. Ratios of 3:1, 15:1, and 63:1 of red-seeded and white-seeded plants, respectively, have been obtained in the F_2 of crosses between red-seeded and white-seeded wheats (*3, 18, 19, 11*). Apparently there may be from one to three factors for red seed color present in different wheats. When one or more of the factors for red are present red seed results.

The results obtained in the present study are given in Table II. Of 364 F_2 plants of the Kubanka No. 8 × Pentad cross that were classified, 295 were red-seeded like the Pentad parent and 69 were white-seeded like the Kubanka No. 8 parent. The observed ratio was 3.21:0.79 with red seed color dominant. There were 599 F_2 plants of the Mindum × Pentad crosses classified. The red-seeded totaled 449 and the white-seeded 150, the ratio being almost exactly 3:1 with red seed color dominant. In both cases the presence of a single factor for seed color is indicated.

TABLE II.—*Classification of F_3 families on the basis of seed color*

Cross.	Family numbers of F_2.	No. of F_3 families.		
		Red-seeded.	White-seeded.	Total.
Kubanka No. 8×Pentad	774 to 792	295	69	364
Mindum×Pentad	793 to 810	247	82	329
Mindum×Pentad	644 to 680	202	68	270

THE F_2 SEED COLOR IN RELATION TO THE RUST REACTION OF THE PROGENY

TABLE III.—*The relation of seed color to the reaction of the F_3 generation of two durum crosses to two biologic forms of P. graminis tritici*

Parents of F_2 families.		Biologic form used.	Reaction of F_2 families.					
			Classes of infection.					
Cross.	Seed color.		Resistant or immune	Near-resistant or near-immune.	Heterozygous.	Near-susceptible.	Susceptible.	Total number of families.
Kubanka No. 8×Pentad	Red	XXXIV.	8	4	70	15	104	201
Do	White	XXXIV.	1	1	14	5	24	45
Mindum×Pentad	Red	I.	23	7	39	6	6	81
Do	White	I.	21	6	43	4	11	85
Do	Red	XXXIV.	9	2	48	10	11	80
Do	White	XXXIV.	5	4	20	1	0	30

Table III summarizes separately the results for the F_3 families arising from red-seeded F_2 parents and for those from white-seeded F_2 parents. The Kubanka 8 × Pentad F_3 families reacted with Form XXXIV as follows: Of those from red-seeded parents, 8 appeared to be resistant, 4 near-

resistant, 70 heterozygous, 15 near-susceptible, and 104 susceptible; of those from white-seeded parents, 1 was classed as resistant, 1 near-resistant, 14 heterozygous, 5 near-susceptible, and 24 susceptible. A brief comparison of these two sets of figures reveals their similarity, that is to say, no correlation of seed color with resistance or susceptibility to rust was indicated.

Mindum × Pentad F_3 families when inoculated with Form I gave the following results: Of those from red-seeded parents, 23 were classed as immune, 7 near-immune, 39 heterozygous, 6 near-susceptible, and 6 susceptible; of those from white-seeded parents, 21 were placed as immune, 6 near-immune, 43 heterozygous, 4 near-susceptible, and 11 susceptible. Here again the distribution of families from red seed was similar to that of the progeny of white-seeded plants. A lack of correlation of seed color with resistance or susceptibility to rust was evident.

Mindum × Pentad F_3 families when tested with Form XXXIV reacted in the following ways: Of those from red-seeded parents, 9 appeared resistant, 2 near-resistant, 48 heterozygous, 10 near-susceptible, and 11 susceptible; of those from white-seeded parents, 5 were classed as resistant, 4 near-resistant, 20 heterozygous, 1 near-susceptible, and none susceptible. A comparison of the distribution of the progeny of the red-seeded parents with that of families arising from white-seeded parents shows differences in the near-susceptible and susceptible classes. These, however, can not be given much weight owing both to the small number of families classified and to the fact that Mindum, the white-seeded parent, was susceptible, whereas only 1 of the 30 families from white-seeded F_2 was in the near-susceptible and susceptible classes. If anything, a negative correlation is indicated.

A summarization of the data concerning the mode of inheritance of rust resistance with respect to seed color shows that no correlation was found to exist between the two. It is important to note that each of the three sets of data shows everal F_3 families which were homozygous for the reaction of the Pentad parent and also homozygous for the seed color of Mindum or Kubanka No. 8 parents. Rust resistance and seed color appeared to be inherited independently.

RESULTS OF INOCULATIONS OF KUBANKA NO. 8 × PENTAD F_3 FAMILIES WITH BIOLOGIC FORM XXXIV.

The data obtained for Kubanka No. 8 × Pentad with Form XXXIV are summarized in Table IV. About 35 control pots of Kubanka No. 8 were inoculated. Of the 308 plants that were infected, 21, or about 7 per cent, had type 1 infections and 287 showed types ranging from x− to 4. In view of the fact that possible errors and variations may result in types of infection outside of the normal range, the type 1 infection on these 21 plants was regarded here as exceptional. The range of infection types on Kubanka No. 8 tested with Form XXXIV was therefore considered to be from x− to 4 (Pl. I, B). All Kubanka No. 8 pots with plants giving types of infection falling within this range were classified as susceptible. Those pots with 9 or more plants, one of which had a type 1 infection, were placed in the near-susceptible class. Pots having a greater percentage of plants with type 1 infections were classed as heterozygous. The distribution of the Kubanka No. 8 pots in the five classes of infection was 5 heterozygous, 5 near-susceptible, and 25 susceptible. There is no reason to believe that 5 of the parent control pots were actually heterozygous

but, rather, the method of classification shows the accuracy with which the determination of pure-line material could be made.

TABLE IV.—*The reaction of Kubanka No. 8×Pentad and F_3 families to biologic Form XXXIV*

Parent varieties and F_3 families.	Class of infection.	Distribution of F_3 plants according to the type of infection shown.							Total of pots of parent varieties or F_3 families.
		0	1	2	X−	3	X+	4	
Kubanka No. 8	Heterozygous	.....	15	....	10	.....	6	14	5
Do.	Near-susceptible	.....	6	....	5	6	14	15	5
Do.	Susceptible	.....	.....	....	56	1	24	136	25
Total		.....	21	....	71	7	44	165	35
Pentad	Resistant	4	270	....	30	.....	.....	.....	30
Do.	Near-resistant	.....	36	....	4	.....	4	.....	4
Do.	Heterozygous	.....	12	....	2	.....	7	.....	2
Total		4	318	....	36	.....	11	.....	36
F_3	Resistant	.....	70	....	.....	.....	.....	.....	9
Do.	Near-resistant	.....	52	....	10	.....	.....	.....	5
Do.	Heterozygous	.....	297	....	183	.....	91	256	84
Do.	Near-susceptible	.....	5	....	30	3	31	112	20
Do.	Susceptible	.....	1	....	108	13	114	1,025	128
Total		.....	425	....	331	16	236	1,393	246

The legitimacy of the foregoing method of analysis will become evident if a brief study is made of the type of data obtained and the object of the work. To make possible a classification which would form an adequate basis for the comparison of the reactions of the F_3 hybrids with those of the parental varieties it was necessary to establish a range for the types of infection normal for each parent. It might appear that the total distribution of types of infection of a parental variety should be considered its normal range, and the hybrid reactions judged accordingly. This would throw all the pots of each parent into a single class, viz, with Kubanka No. 8 there would be 35 pots in the susceptible class, which would be the same as ignoring the possibility of any error in the results. Obviously that would lead to inaccuracies in classifying the hybrids, for it is well established that even with carefully controlled conditions a certain amount of error is unavoidable. The method used has on the contrary much in its favor, for it tends to throw all doubtful F_3 families into the near-susceptible, heterozygous, or near-resistant classes.

Thirty-six control pots of Pentad were inoculated. Of the 369 plants that were infected, all but 11 ranged from 0 to X− in type of infection. These 11 showed infection of the X+ type. Classification of the pots on the same basis as was used in the case of Kubanka No. 8 resulted in 30 being placed as resistant, 4 near-resistant, and 2 heterozygous (Pl. I, A).

The use of these terms to designate the different classes of infection is relative. It is convenient to call one parent susceptible and the other resistant regardless of whether the susceptibility or resistance is partial or complete. For the purpose of studying the genetics of the inheritance

of the rust reaction it is immaterial just what the classes are called. In the present cross, the Kubanka No. 8 parent is only partially susceptible but the Pentad parent is quite resistant. It thus happens that the ranges for the two parent varieties overlap somewhat at the x− type of infection.

A total of 246 F_3 families were tested. The number of F_3 plants that showed infection was 2,401. The distribution of F_3 plants according to their types of infection and to the susceptible classes of the various families to which they belonged is given in Table IV. The 246 F_3 families were classified as 9 resistant, 5 near-resistant, 84 heterozygous, 20 near-susceptible, and 128 susceptible (Pl. I, C–E). Then, one-sixth of the pots of Pentad, the resistant parent, were classed other than resistant. Considering the F_3 on this basis, there were 9 plus 1.5, or 10.5 families which were as resistant as the resistant parent. The ratio of this number to that of the remaining families is 1:22.4. The presence of 2 factors for rust reaction fairly satisfactorily explains the results. There was no evidence that either susceptibility or resistance was dominant, for the F_3 plants in the heterozygous class occurred in approximately equal numbers on either side of the x− type.

RESULTS OF INOCULATIONS OF MINDUM × PENTAD F_3 FAMILIES WITH BIOLOGIC FORM I

The data obtained with Form I are summarized in Table V. Twenty-three control pots of each of the parental varieties were inoculated. Of the 274 Mindum plants that were infected, 269 had 0 and 1 types of infection with a killing of the leaf tips in most cases, and 5 had type 4 infections. These 5 plants clearly were either not Mindum or else they had been infected by chance spores of some other form. Accidental infection is almost unavoidable where many rust forms are being worked with but fortunately it is of infrequent occurrence and usually can be detected.

TABLE V.—*The reaction of the F_3 of Mindum × Pentad to biologic Form I*

Parent varieties or F_3 families.	Class of infection.	Distribution of F_3 plants according to the type of infection shown.							Total pots of parent varieties or F_3 families.
		0	1	2	x−	3	x+	4	
Pentad	Heterozygous	13	4	....	9	1	5	.....	4
Do.	Near-susceptible	1	2	....	6	3	10	.....	3
Do.	Susceptible	.....	.....	....	10	13	14	76	16
Total		14	6	....	25	17	29	76	23
Mindum	Immune	236	12	....	.....	.....	.....	.....	20
Do.	Near-immune	15	.....	.....	.....	.....	.....	2	2
Do.	Heterozygous	6	.....	.....	.....	.....	.....	3	1
Total		257	12	....	.....	.....	.....	5	23
F_3	Immune	603	.....	.....	.....	.....	.....	.....	44
Do.	Near-immune	158	.....	.....	.....	7	1	5	13
Do.	Heterozygous	723	9	2	16	60	22	190	82
Do.	Near-susceptible	10	3	....	10	22	15	68	10
Do.	Susceptible	.....	.....	.....	6	72	2	165	17
Total		1,494	12	2	32	161	40	428	166

The range of infection for Mindum was considered to include 0 and 1 types of infection. In the immune class, 236 of the 248 plants had 0 type infections, hence the immunity of Mindum to Form I is evident. The pots of Mindum were classified as 20 immune, 2 near-immune, and 1 heterozygous (Pl. 2).

In the 23 control pots of Pentad 167 plants were infected. Of these, 147 had types of infection ranging from x− to 4, and 20 showed types 0 and 1. Leaving these 20 plants out of consideration, the range of the type of infection was from x− to 4, as was the case with Kubanka No. 8 when inoculated with Form XXXIV. At first glance it would seem that 20 is a rather large number of plants to leave out of consideration in a population of 167. It is not as significant as it appears to be, however, for many of the plants with 0 type infections showed only a few vague necrotic flecks which were not clearly distinguishable from similar effects sometimes produced on seedlings as a result of environmental conditions. The classification of Pentad pots gave 16 as susceptible, 3 near-susceptible, and 4 heterozygous (Pl. 2).

A total of 166 F_3 families were tested. The number of F_3 plants inoculated was 2,340. Of these 2,169 showed infection. The distribution of F_3 plants according to their types of infection and to the classes of the various families to which they belonged is given in Table V. The 166 F_3 families were classified as 44 immune, 13 near-immune, 82 heterozygous, 10 near-susceptible, and 17 susceptible (Pl. 2).

A larger proportion of families was classified as immune and near-immune than as susceptible and near-susceptible. This was to be expected from the progeny of the parents, one of which was immune and the other only moderately susceptible. Approximately one-eighth of the pots of Mindum, the immune parent, fell outside the immune class. The F_3 progeny, considered on this basis, shows 44 plus 5.5, or a total of 49.5 families which appeared to be as immune as the immune parent. The ratio of 49.5 to 166, which is the total number of families classified, would seem to indicate the presence of a single differential factor. The variable results obtained with the Pentad parent prevent a more detailed analysis.

The distribution of plants in the heterozygous class with respect to the various types of infection shows a total of 734 with 0, 1, and 2 types of infection (723 being of the 0 type) and 288 with types ranging from x− to 4. The dominance of immunity is evident.

RESULTS OF INOCULATIONS OF MINDUM×PENTAD F_3 FAMILIES WITH BIOLOGIC FORM XXXIV

The results obtained are summarized in Table VI. Thirty-three control pots of each of the parental varieties were inoculated. The reactions of Mindum and Pentad to Form 34 were the reciprocals of their reactions to Form I. Of the 375 Mindum plants that showed infection all but 9 had x+ and 4 types of infection. The remaining 9 plants had x− and 3 types of infection. Mindum is here the susceptible parent with a range of types of infection which is narrower than that of the susceptible parents in the work previously discussed. The pots of Mindum were classified as 2 heterozygous, 4 near-susceptible, and 27 susceptible (Pl. 3).

TABLE VI.—*The reaction of the parent varieties and F_2 families of Mindum×Pentad o biologic Form XXXIV.*

Parent varieties or F_2 families.	Class of infection.	Distribution of F_2 plants according to the type of infection shown.							Total pots of parent varieties or F_2 families.
		0	1	2	X−	3	X+	4	
Mindum	Heterozygous				3	2	2	14	2
Do	Near-susceptible				1	3	7	31	4
Do	Susceptible						39	273	27
Total					4	5	48	318	33
Pentad	Resistant	37	115	3	131	2			26
Do	Near-resistant	3	18		24		5		5
Do	Heterozygous		2		9	3	4	1	2
Total		40	135	3	164	5	9	1	33
F_3	Resistant	38	53	8	95				14
Do	Near-resistant	12	29	1	53		8		6
Do	Heterozygous	38	48	8	400	144	232	139	68
Do	Near-susceptible				8	4	47	90	11
Do	Susceptible					1	39	109	11
Total		88	130	17	556	149	326	338	110

Infection appeared on 357 of the inoculated Pentad plants. Of these 9 reacted with x+ type infections and 1 gave a 4 type. The other 347 Pentad plants gave a range of infection types from 0 to 3. Only 5 plants showed type 3 infections, therefore this type would appear not to belong to the range for the resistant parent. However, the type 3 infection is much more like the x− type than like the x+. In fact, experiments indicate that a variety of which the seedling plants give type 3 infection in the greenhouse is resistant in the field, whereas varieties showing x+ in the greenhouse probably are not resistant in the field. Consequently, the 3 type infection may well be included in the range of the Pentad parent. Classification of Pentad pots gave 26 resistant, 5 near-resistant and 2 heterozygous (Pl. 3).

A comparison of the results obtained for Pentad in the study with the Kubanka No. 8 × Pentad cross with those obtained here reveals considerable differences in the number of plants showing the various types of infection. The two series of inoculations were made at different periods, and consequently the conditions were not comparable. This strikingly brings out the fact that the value of the data taken on the hybrid material lays in the direct comparison made with the results from pots of parental plants grown under identical conditions. Reactions which, under one set of circumstances, would place an F_3 family in the near-resistant class might, under other circumstances, cause it to be classed as heterozygous, depending on the parental reactions at each period.

It was noticeable that both parent and hybrid plants showed an abundance of hypersensitiveness to Form XXXIV. This probably accounts in great measure for the appearance of such large numbers of x− and x+ types of infection. Sharp hypersensitive areas occurred around a large proportion of the uredinia of types 3 and 4 infections. Usually type 4 shows

very little chlorosis and no necrosis. Here, however, about 50 per cent of the type 4 uredinia showed such a decided necrotic bordering that the advisability of creating a special class for this type of infection was considered. Eventually it was concluded that the establishment of an additional type would possibly lead to some confusion without really proving of value, as it was apparent that plants infected in this manner were susceptible, on account of the development of numerous large uredinia.

A total of 110 F_3 families were tested. There were 1,711 F_3 plants inoculated, of which 1,604 showed infection. The distribution of F_3 plants is summarized in Table VI. Classification of the 110 F_3 families gave 14 as resistant, 6 near-resistant, 68 heterozygous, 11 near-susceptible, and 11 susceptible (Pl. 3). Since between a third and a fourth of the Pentad pots were placed outside of the resistant class, it may be considered that 14 plus 4 or 18 F_3 families were as resistant as the resistant parent. This number of resistant families is too small to indicate definitely the presence of a single factor, and it is much too large to make a two-factor hypothesis feasible. The single factor is the more plausible explanation.

If the near-resistant and resistant classes are grouped together, and similarly, the near-susceptible and susceptible classes, a fairly satisfactory 1:2:1 ratio is obtained. The presence of a single factor would appear probable in this case. The results do not make possible a more exact genetic analysis. Examination of the distribution of F_3 plants in the heterozygous class showed a lack of dominance for either susceptibility or resistance.

COMBINATIONS OF SUSCEPTIBILITY AND RESISTANCE OF MINDUM × PENTAD F_3 FAMILIES TO FORM I AND FORM XXXIV

Table VII correlates the results obtained with Form I and Form XXXIV for those families which were inoculated with both forms. Various combinations of susceptibility, resistance, and heterozygosity to the two biologic forms appear. Six F_3 families were definitely resistant to Form XXXIV and immune from Form I as, for example, 809-21, 805-16, and 796-4 (Pl. 4, A, B, C). Two families were quite susceptible to both forms, as 804-7 (Pl. 4, E). Other families were resistant to or immune from one form and susceptible to the other. Still other F_3 families were resistant or susceptible to one form and heterozygous to the other, as 806-24 (Pl. 4, D). Many families were heterozygous to both forms. There were also combinations of near-resistance and near-susceptibility, as 809-8 (Pl. 4, F).

TABLE VII.—*The classified reaction of Mindum × Pentad F_3 families to biologic Forms I and XXXIV*

Reaction to Form XXXIV.	Reaction to Form I.					Total.
	Immune.	Near-immune.	Heterozygous.	Near-susceptible.	Susceptible.	
Resistant	6	0	8	0	0	14
Near-resistant	1	0	2	1	1	5
Heterozygous	14	7	36	3	7	67
Near-susceptible	3	1	5	1	1	11
Susceptible	3	1	3	1	2	10
Total	27	9	54	6	11	107

The most significant feature of Table VII is that six F_3 hybrid families are shown to have been definitely resistant to both biologic Forms I and XXXIV. Considering the reaction of the parental varieties to these biologic forms, it is very likely that at least one of the two near-resistant families was likewise homozygous in its reaction. On this basis it would seem that two main differential factors may explain the results obtained. These factors, if present, are inherited independently, which makes possible the combining of resistance to both forms in a single variety.

THE REACTION OF THE MINDUM × PENTAD F_3 FAMILIES FROM WHITE-SEEDED PARENTS TO FORM I AND FORM XXXIV

Table VIII correlates the results obtained for F_3 families from white-seeded parents when inoculated with Forms I and XXXIV. Out of a total of 34 families 2 were resistant to Form XXXIV and immune from Form I. The indications of 3: 1 ratios for Forms I and XXXIV are here substantiated in a very close 15: 1 ratio.

TABLE VIII.—*The classified reaction of the progeny of Mindum × Pentad white-seeded F_2 plants to biologic Forms I and XXXIV*

Reaction to Form XXXIV.	Reaction to Form I.					Total.
	Immune.	Near-immune.	Heterozygous.	Near-susceptible.	Susceptible.	
Resistant	2	0	3	0	0	5
Near-resistant	1	0	2	0	2	5
Heterozygous	3	2	11	1	2	19
Near-susceptible	0	0	0	0	1	1
Susceptible	0	0	0	0	0	0
Total	6	2	16	1	5	30

The object of giving special treatment to the F_3 progeny of white-seeded F_2 plants is a recognition of the fact that the economic aspects of a problem warrant careful consideration. Pentad, although high in rust resistance, is a poor milling wheat. Owing to the difficulty of distinguishing the thrashed grain of one red durum from another, millers have come to discriminate against all red durums. There is consequently no immediate future for a new rust-resistant red durum, even though it be of good milling quality.

DISCUSSION

The study of the reaction of the F_3 generation of the durum crosses Mindum × Pentad and Kubanka No. 8 × Pentad to two biologic forms of *P. graminis tritici* has shown that all combinations of susceptibility and resistance to these forms may appear. The reaction of Kubanka No. 8 × Pentad F_3 families to Form XXXIV indicated the presence of two factors with neither susceptibility nor resistance dominant. The results obtained from Mindum × Pentad F_3 families inoculated with Form I can be explained on the basis of a single main factor differenc with immunity dominant. The data for the reaction of Mindum × Penta

F_3 families to Form XXXIV gave some indication that a single factor was present, but dominance was not apparent. No evidence of a correlation between seed color and rust reaction was found.

As the action of each biologic form in general was fairly uniform on individual plants of the differential varieties and also on the plants of Kubanka No. 8, Mindum and Pentad and the F_3 families, barring the x− and x+ types of infection, it may be concluded that the urediniospores of each form probably were of the same genetic constitution. Therefore, the factors concerned with susceptibility and resistance must have been located in the plants themselves. Furthermore, as different biologic forms differ in their parasitizing capabilities on the same varieties of wheat, their genetic constitution can not be the same. It is probable, then, that factors governing susceptibility and resistance to different biologic forms used in this experiment are different in nature and are located in different chromosome pairs. The results of the present investigation show that when two varieties of wheat react reciprocally to two biologic forms of *P. graminis tritici*, one variety being resistant to one form and the other to the other form, it is possible by means of crossing these varieties to obtain progeny resistant to both forms of rust. These results furnish further evidence for the belief that eventually a variety of wheat can be produced which will be resistant to all biologic forms of this rust.

SUMMARY

(1) A study was made of the parasitic capabilities of two biologic forms of *Puccinia graminis tritici* on the F_3 progeny from crosses between three varieties of *Triticum durum*.

(2) Two of the parental varieties, Mindum and Pentad, reacted reciprocally to two of the biologic forms used.

(3) The constancy of the parasitic capabilities of the biologic forms had been determined previously and, as additional evidence, complete sets of differential varieties were inoculated at the commencement and at the completion of the work with each form. Form XXXIV attacked Kubanka No. 8 but had little effect on Pentad. Form I produced no uredinia on Mindum but developed vigorously on Pentad. On the other hand, Form XXXIV infected Mindum severely but developed weakly on Pentad.

(4) A separate set of 8 to 20 plants from each F_3 family was grown for use with each form of rust. Only first leaves were inoculated.

(5) With respect to the reaction of the F_3 families to each of the two biologic forms of rust there were some families that were as resistant as the resistant parent, some as susceptible as the susceptible parent, and others which were heterozygous.

(6) The results of using Form XXXIV on Kubanka 8 × Pentad F_3 families indicated the presence of two differential factors.

(7) A single main factor difference explained fairly satisfactorily the results obtained for Mindum × Pentad F_3 families inoculated with Form I.

(8) The results obtained when Mindum × Pentad F_3 families were infected with Form XXXIV, gave some indication of the presence of a single factor. The data would not permit, however, a definite genetic analysis.

(9) No relation was found between seed color and rust resistance.

(10) All combinations of susceptibility and resistance of individual Mindum × Pentad F_3 families to Form I and Form XXXIV appeared. Out of a total of 110 F_3 families 6 were highly resistant to both forms of rust and 2 of these families were from white-seeded parents.

(11) With the varieties of wheat used, it was found possible to combine, in a single variety, resistance to two biologic forms of stem rust of wheat, when crosses were made between two varieties which reacted reciprocally to these rust forms.

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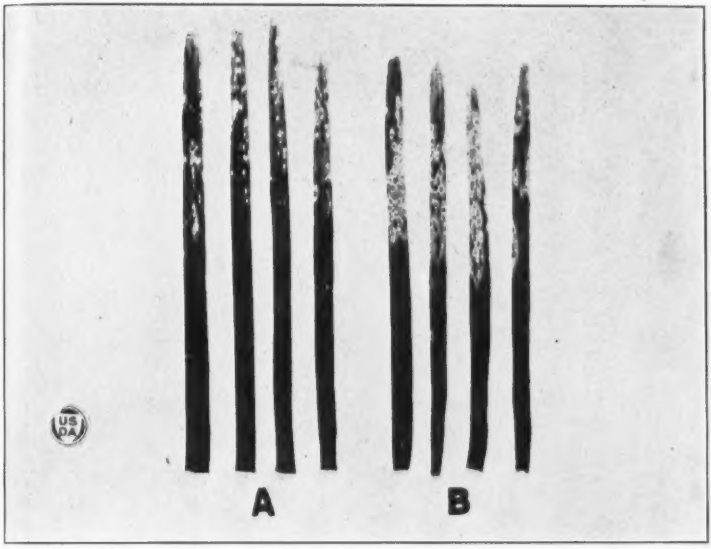
PLATE 1

The reaction of Pentad, Kubanka No. 8, and F_3 hybrids to biologic Form XXXIV.

A.—Pentad, the resistant parent, normally giving the o-type of infection with an occasional uredinium.

B.—Kubanka No. 8, the susceptible parent, giving numerous uredinia more or less surrounded by chlorotic areas.

C, D, E.—Typical F_3 families that appeared resistant, heterozygous, and susceptible, respectively.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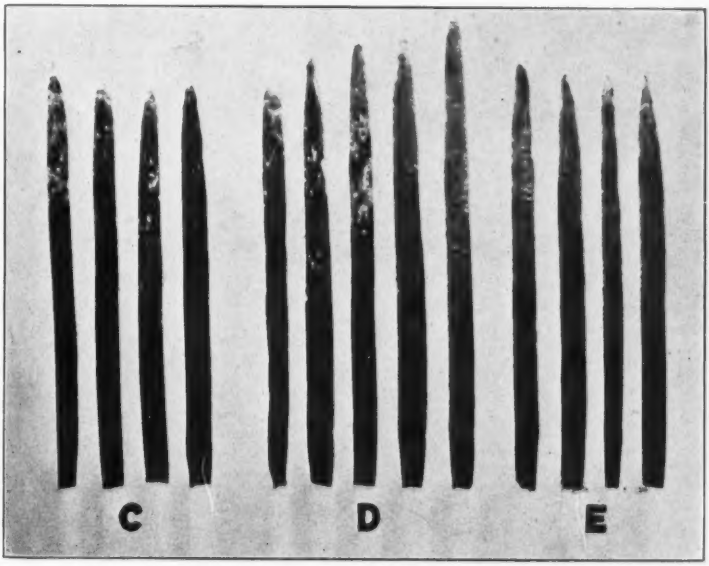
B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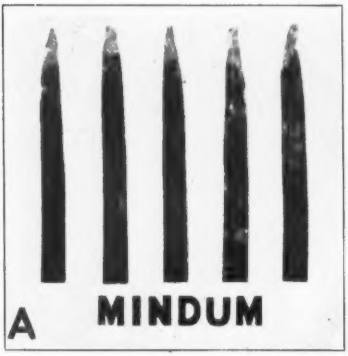
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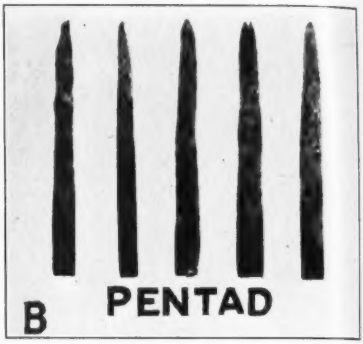
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PENTAD

C
SEGREGATING F3

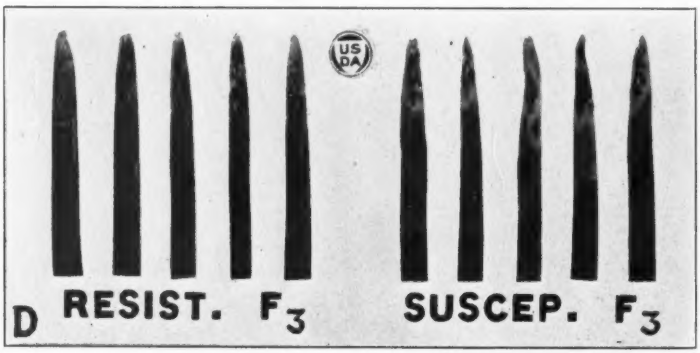

USDA
D
RESIST. F3
SUSCEP. F3

PLATE 2

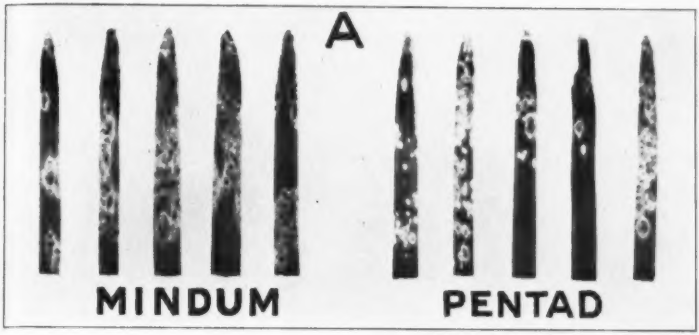
The reaction of Mindum, Pentad, and F_3 hybrids to biologic Form I.
A.—Mindum is immune, normally giving a few hypersensitive flecks.
B.—Pentad is susceptible with types of infection varying from x− to 4.
C, D.—The F_3 families shown are, respectively: 802–6, which appeared heterozygous; 809–5, which was immune like Mindum; 804–17, which showed greater susceptibility than Pentad.

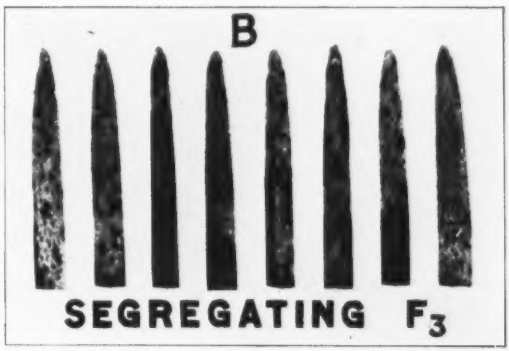
PLATE 3

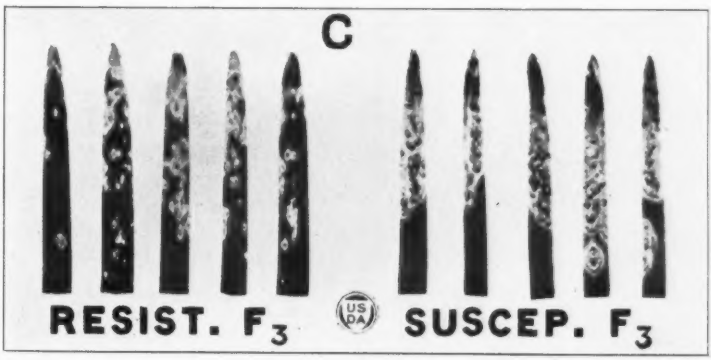
The reaction of Mindum, Pentad, and F_2 hybrids to biologic Form XXXIV.

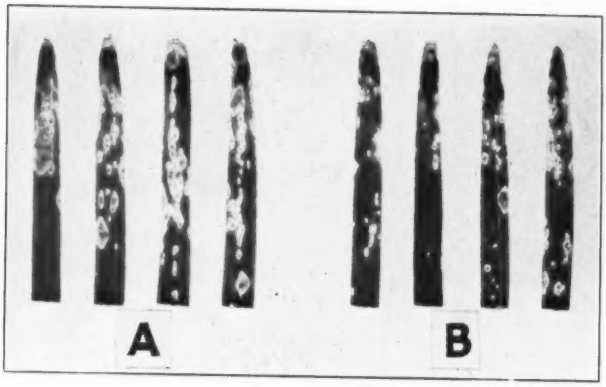
A.—Mindum, the susceptible parent, normally giving x+ and 4 types of infection, principally the latter; Pentad, the resistant parent, with a range of infection types from 0 to x−.

B, C.—The F_2 families shown are, respectively: 807–18, heterozygous; 805–16, as resistant as the resistant parent; 804–17, susceptible like Mindum.


A
MINDUM
PENTAD


B
SEGREGATING F3


C
RESIST. F3
USDA
SUSCEP. F3


A
B

C

D

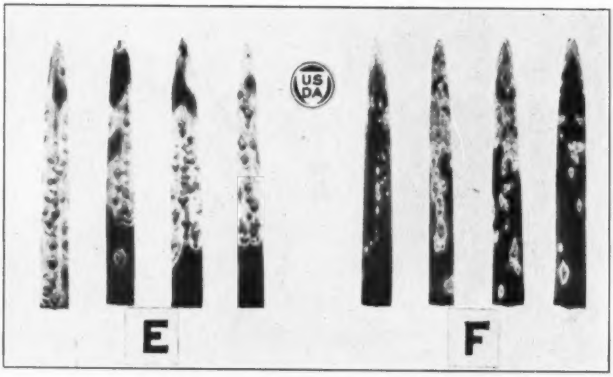

USDA
E
F

PLATE 4

The reaction of certain Mindum×Pentad F_3 families to biologic Form XXXIV.

A, B.—Two F_3 families (809–21 and 805–16, respectively) from white-seeded parents, immune from Form I and resistant to Form XXXIV.

C.—Family 796–4, from red-seeded parents and similar in reaction to A and B.

D.—Family 806–24, which was heterozygous to Form I and resistant to Form XXXIV.

E.—Family 804–7, which was highly susceptible to both forms of rust.

F.—Family 809–8, which fell into the near-susceptible class with Form I and in the near-resistant class with Form XXXIV.

A STUDY OF RUST RESISTANCE IN A CROSS BETWEEN MARQUIS AND KOTA WHEATS [1]

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INTRODUCTION

A knowledge of the mode of inheritance of certain plant characters is essential if the breeder is to outline his particular problem. Accordingly one of the first steps in the investigations carried on for the purpose of producing rust-resistant spring wheats has been to determine the genetic possibilities of attaining the desired end.

Several years ago it was a common belief that the rust organism changed its infection capabilities so rapidly as to seriously interfere with the production of rust-resistant wheat. The investigations of Stakman and his coworkers (*6*, 7, and *8*)[2] at the Minnesota station have proved that the variable results which have been obtained under field conditions are due to numerous biologic forms of rust which can only be differentiated by their manner of infecting particular pure lines of wheat. This has led naturally to the survey to determine the prevalence of particular rust forms and to the use of these forms in breeding experiments. The method is to determine those wheat varieties which are resistant to particular rust forms and then by means of crosses to build up desirable rust-resistant varieties.

Kota (*9*) is the only rust-resistant spring common wheat which so far has been discovered, except for certain recently produced hybrids. Under humid conditions, however, Kota does not yield as well as Marquis and is seriously lacking in strength of straw.

The purpose of this paper is to present certain studies of a cross between Kota and Marquis which were carried on with the hope of solving the following questions:

1. Is the resistance of Kota to different biologic forms due to a single genetic factor, as in the case of Kanred?

2. Given Kota and Marquis, which react reciprocally to two biologic forms of rust, is it possible to produce from a cross of Kota and Marquis a variety resistant to both biologic forms?

3. To determine the possibility of using the reaction of F_3 seedlings in the greenhouse to particular biologic forms of stem rust as a means of isolating F_3 families which have the resistance of the Kota parent under field conditions.

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[2] Reference is made by number (italic) to "Literature cited," p. 1012.

THE PRESENT STATUS OF THE PROBLEM

For reviews of earlier studies which have led to the present mode of attack, the reader is referred to previous papers (*3*, *4*). In the present paper it seems sufficient to sum up the facts now at hand without making an extended review of literature.

Thirty-seven biologic forms of stem rust have been discovered (*6*), and of these 21 have been found in the upper Mississippi Valley. If the resistance of a wheat variety to each of these biologic forms was due to a separate set of independently inherited factors the problem would be a very difficult one. If the resistance to one biologic form was due to a genetic factor which was allelomorphic to a factor for resistance to a second form the problem of obtaining a wheat resistant to all biologic forms would be an impossibility. No variety of common wheat has been found which is resistant to all biologic forms of rust; but Khapli, an early maturing emmer, is resistant to all 37 forms.

Crosses of Khapli with common varieties have given self-sterile F_1 progeny. This has led to the attempt to transfer the resistance of Khapli to a durum variety, with the hope of then transferring this resistance to common wheats by a subsequent cross.

That the resistance of durum wheats can be transferred to common wheats has been shown. Several years ago a cross was made between Iumillo and Marquis. There was found to be a strong linkage between the durum characters and resistance although this was overcome by growing large numbers. Several plants were obtained from an F_3 population of from 20,000 to 30,000 plants which appeared to have the spike characters of common wheat and which also appeared rust resistant. Selection has been practiced further and the wheats thus obtained appear of desirable field type and have shown high resistance in field experiments.

Kanred, a rust-resistant winter wheat, produced at the Kansas station, proved immune from 11 of the 21 biologic forms of rust which are found in the North-Central States. This immunity was transferred to spring wheats by crosses between Kanred and Marquis. The immunity from all 11 biologic forms was apparently due to a single genetic factor (*1*, *2*).

Puttick (*5*), in a study of the F_2 progeny of a cross between Mindum and Marquis, obtained seedling wheats which combined the resistance of both parents. In this study, two biologic forms of rust were used to which Marquis and Mindum reacted reciprocally, one parent being resistant and the other susceptible to the respective rust forms. Puttick, however, did not grow the seedlings to maturity so it was impossible to say whether they were of common or durum types.

In the paper preceding this, Harrington and Aamodt (*2a*) have presented the results of a cross between Pentad, which is resistant to biologic Form XXXIV and susceptible to biologic Form I, and Mindum, which reacts in a reciprocal way to these rust forms. The resistance of Pentad to Form XXXIV and of Mindum to Form I appears to be due to a single main genetic factor in each case. These two factors appear to be independently inherited and in the production, from the cross Pentad × Mindum, of a wheat which is resistant to both biologic forms, it is only necessary to grow enough plants to obtain the necessary recombination of the two factors for resistance.

The brief outline given shows the present status of the problem, the mode of attack being to build up synthetically, by certain crosses, wheats which are resistant to as many biologic forms as possible. For this mode of attack a knowledge of the number and nature of the inherited factors is essential.

MATERIAL AND METHODS

Certain general methods of work have been used as in previous studies. For this reason only brief descriptions will be made.

Pure lines of the parental varieties were used in making the crosses. The F_1 generation plants were grown in the greenhouses[3] at Washington, D. C., during the winter of 1920–21 and the F_2 generation was grown during the summer of 1921. The season was a very unfavorable one and the seed produced was rather badly shrivelled in certain cases. Individual plant data on spike characters, average seed length, seed plumpness, and seed texture were taken and the F_3 seedlings obtained from sowing seed of individual F_2 plants were inoculated in the greenhouse with the rust forms chosen.

In deciding which rust forms [4] to use, a study was made of the degrees of infection of seedlings of Marquis and Kota when inoculated at the same time and handled in a similar manner.

Eleven biologic forms were available in the greenhouse and all were used in inoculations for the purpose of discovering two to which the two wheat varieties reacted reciprocally. The reactions obtained on Marquis and Kota are given in Table I. Although Kota is rather highly resistant as tested under field conditions, the difference in seedling infection of Marquis and Kota in the greenhouse is not very great. In nearly all cases, however, where there is a difference, Kota shows a lower degree of infection than Marquis. The meaning of the symbols used to designate the types or degrees of infection is given after Table I.

TABLE I.—*Reaction of Kota and Marquis to 11 forms of stem rust, expressed by type of infection produced*

Variety.	Type of infection produced by biologic form of rust No.—										
	I.	III.	IX.	XVII.	XVIII.	XIX.	XXI.	XXVII.	XXIX.	XXXII.	XXXIV.
Marquis	4	4–	3	4	4	1 and 2	4	2+	4	3+	3+
Kota	3+	3+	3	4–	3	3	3+	0	3+	3	3

[3] The writers are indebted to Mr. J. Allen Clark, of the Office of Cereal Investigations, for growing the F_1 generation and returning seed of the same.

[4] The biologic forms used were obtained from M. N. Levine, who kindly made them available for our use

Explanation of classes of host reaction and of corresponding types of infection resulting from inoculation of seedling wheat plants with spores of Puccinia graminis tritici [6]

Classes of host reaction.	Types of infection.
Immune (immune in the field).....	0. No uredinia developed but definite hypersensitive areas present.
Very resistant (highly resistant under field conditions).....	1. Uredinia minute and isolated; surrounded by sharply-defined, continuous, hypersensitive areas; hypersensitive areas lacking uredinia also may be present.
Moderately resistant (highly resistant under field conditions).....	2. Uredinia isolated and small to midsized; hypersensitiveness present in the form of necrotic areas in circles; uredinia often surrounded by green islands.
Moderately susceptible (somewhat resistant in the field).....	3. Uredinia midsized; coalescence infrequent; development of rust somewhat subnormal; true hypersensitiveness absent; chlorotic areas, however, may be present.
Very susceptible (entirely susceptible under field conditions).....	4. Uredinia large or varying from midsized to large, numerous and confluent; true hypersensitiveness entirely absent, chlorosis seldom present.

The studies which have been made show that a pure-line wheat variety inoculated with a single biologic form frequently shows a variation in infection from type 3 to type 4 and occasionally from type 2 to type 3, due to varying environmental conditions to which the seedlings are exposed, even when the conditions of the greenhouse test are kept as uniform as possible. These variations apparently are not due to changes in the genotype.

The most striking differences in the infection of Kota and Marquis were obtained with Form XIX and Form XXVII and accordingly these two forms were chosen for the study. Form XIX was collected in Pocatello, Idaho, in November, 1918, and has been cultured through approximately 51 urediniospore generations. The Form XIX used to inoculate the F_4 material was collected in North Dakota in July, 1921, and has been cultured through approximately 17 generations. Form XXVII was collected in Barges, France, in August, 1919, and has been cultured through approximately 45 urediniospore generations.

Between 15 and 20 seeds of each individual F_2 plant were sown in a single pot, but, owing to the unfavorable conditions under which the F_2 plants were grown, the germination of these seeds was low. No pots were used in the study in which the number of F_3 plants per pot fell below 8. While 8 plants is much too small a number to prove absolutely the genetic nature of the individual F_2 parent, it gives a fair indication of the genetic class in question. Larger numbers of seeds could not have been used without depleting the quantity of seed reserved for sowing to obtain F_3 families under field conditions. The results obtained with the small numbers of seed used are reliable enough, however, to furnish a good idea of the approximate number of factors involved, and, from the plant-breeding standpoint, the information obtained is sufficient to answer the main questions for which the investigation was undertaken. It is recognized that from the genetic standpoint larger numbers of seedlings in each F_3 family should be inoculated.

[6] These descriptions of types of infection in the greenhouse are based on those given by Stakman and Levine (6).

One of the problems which it was hoped to solve was the discovery of a greenhouse test of F_3 seedlings which would give an accurate estimate of the behavior of Marquis × Kota.F_3 families under field conditions. As no such test was available, the larger part of the Marquis × Kota material was grown in the rust nursery and an artificial epidemic of stem rust induced by the use of the available biologic forms of stem rust which were present in the greenhouse and which were known to have been found in the spring-wheat region. The forms of rust used for the field epidemic in 1922 are those listed in Table 1, with the exception of Form XXVII, which was originally collected in Barges, France, and Form XXXIV which was collected in South Africa. These two forms were not used for the nursery epidemic.

Between 25 and 50 plants were grown in the field nursery from each selected F_3 family. The rust infection was heavy on susceptible types, as shown by the infection on Marquis, and consequently the determination of those F_3 families which had the Kota type of resistance to the nine biologic forms in question could be made with accuracy.

EXPERIMENTAL RESULTS

DWARF PLANTS OBTAINED IN THE F_2 PROGENY

The F_2 material of the Marquis × Kota cross grown in the plant-breeding nursery in 1921 was not severely infected with stem rust and, therefore, was used for the greenhouse studies. Considerable material also was grown in the rust nursery. With most hybrid material in which Kota had been used as one parent, dwarf plants appeared in the F_3 generation. The frequency of the appearance of such plants was determined for the F_2 progeny which grew in the rust nursery in 1921. Of a total of 787 F_2 plants, 666 were of normal type and 121 were dwarfs. Seed from some of these dwarfs was sown in the greenhouse and only dwarf plants resulted. The results may be explained genetically by the assumption that one of the parents contains two factors, one for dwarf habit and the other which inhibits the expression of the dwarf factor. On this basis a 13:3 ratio of normals and dwarfs is expected in F_3. The number of dwarfs expected in a population of 787 would be 147. The number of dwarf plants obtained was somewhat less than the expectation for a 13:3 ratio.

THE AWNLESS OR BEARDED CHARACTER IN RELATION TO SEED CHARACTERS

Kota is a bearded wheat, while Marquis has short apical awns and is commonly classified as awnless. The F_1 hybrids have somewhat longer apical awns than Marquis. At harvest time some of these tip awns were broken off and it was impossible to separate the awnless from the hybrid types with certainty. For this reason awnless and apically-awned (hybrid) forms were placed together. In an earlier study of a cross between Marquis and Preston, experimental data gave reason for the belief that the awn was an important physiological organ under the conditions of the experiment. Accordingly a somewhat similar study was made of the Kota × Marquis plants (Tables II, III, IV).

TABLE II.—*Distribution of F_2 plants of the cross between Marquis and Kota into classes according to average length of seed, in relation to plant and spike characters*

Plant type.	Classes for seed length (in millimeters).						Total plants.	Mean.
	4.8	5.1	5.4	5.7	6.0	6.3		
Normal:								
Awnless	.....	12	72	174	114	12	384	5.73±0.01
Bearded	.....	2	11	51	64	9	137	5.85±0.01
Dwarf:								
Awnless	5	9	5	3	1	1	24	5.26±0.07
Bearded	3	3	6	.....	.....	.....	12	5.18±0.05

TABLE III.—*Distribution of F_2 plants of the cross between Marquis and Kota into classes according to the percentage of hardness of seed, in relation to plant and spike characters*

Plant type.	Classes for percentage of hardness (100=corneous).								Total plants.	Mean.
	40	45	50	55	60	65	70	75		
Normal:										
Awnless	2	....	53	119	162	24	23	1	384	58.92±0.18
Bearded	....	1	24	51	46	10	5	....	137	57.01±0.29
Dwarf:										
Awnless	.....	.....	13	11	7	2	.....	.....	33	54.70±0.54
Bearded	.....	.....	3	8	3	.....	.....	.....	14	55.00±0.04

TABLE IV.—*Distribution of F_2 plants of the cross between Marquis and Kota into classes according to the percentage of plumpness of seed, in relation to plant and spike characters*

Plant type.	Classes for percentage of plumpness.												Total plants.	Mean.
	30	40	45	50	55	60	65	70	75	80	85	90		
Normal:														
Awnless	2	2	...	13	...	30	14	82	115	36	86	4	384	74.02±0.33
Bearded	...	...	...	1	...	9	6	31	33	33	18	6	137	75.44±0.44
Dwarf:														
Awnless	...	5	1	11	1	4	2	4	5	...	...	...	33	56.82±1.38
Bearded	...	...	1	3	1	1	2	5	1	...	...	...	14	61.79±1.73

Apparently the presence of the awn results in the production of a somewhat longer seed on the normal plants, the average difference being about 0.1 mm. The texture of the seeds of the awnless normal plants was somewhat harder than that of those on the bearded plants. The percentage of plumpness of the seed of the bearded normal plants averaged slightly higher than in the case of the awnless plants although, in the light of the probable error, the difference obtained is not significant. In the determinations of texture, 100 is the standard for completely corneous seed, the border line between subcorneous and soft being around 50. The percentage of plumpness of seed is taken in a somewhat similar manner.

REACTION OF F_3 SEEDLINGS AND THEIR PARENT VARIETIES TO BIOLOGIC FORMS XIX AND XXVII

Pots of the parent varieties, Marquis and Kota, inoculated and handled in the same manner as the hybrid F_3 families, were used as controls. In order to assist in the study the parents and the various F_3 families were classified into groups on the basis of their resistance or susceptibility to infection by these two forms of rust as follows:

Classes of resistance and susceptibility to infection in the Marquis-Kota cross when F_3 seedling families and the parent varieties were inoculated with biologic Forms XIX and XXVII in the greenhouse in the winter of 1921–22

BIOLOGIC FORM XIX

IR=Resistant in classes of infection types 1 to 2, or that of the Marquis parent.
HR=Heterozygous with the Marquis or types 1 and 2 reaction dominant.
H=Heterozygous with the Kota or type 3 reaction dominant.
HS=Reaction mainly of the Kota type, type 3, with an occasional plant of the Marquis type of reaction.
IS=Reaction of type 3, like the Kota parent.

BIOLOGIC FORM XXVII

R=Infection of type 0, or the immunity of the Kota parent.
IR=Reaction of type 2, the resistance of the Marquis parent. The uredinia were somewhat larger than for the normal type 2 infection.
H1=Heterozygous with reactions of types 0 and 2.
H2=Heterozygous with reactions of types 0, 2, and 4.
H3=Heterozygous with reactions of types 2 and 4.
H4=Heterozygous with reactions of types 0 and 4.
S=Susceptible with reaction in type 4 only.

The results obtained from inoculating the parents and F_3 hybrids with biologic Form XIX are given in Table V.

TABLE V.—*Distribution of F_3 seedlings of the cross between Kota and Marquis according to types of infection caused by biologic Form XIX*

Classes of parents and F_3 crosses.	Distribution by types of infection.										Number of plants.	Number of families.
	0	−1	1	1+	−2	2	2+	−3	3	3+		
F_3 families IR	4	17	170	18	14	150	39	.....	.....	....	412	40
F_3 families HR	9	1	147	15	13	163	15	13	101	1	478	44
F_3 families H	23	....	118	8	15	187	27	123	768	....	1,269	114
F_3 families HS	7	1	20	3	....	17	3	45	381	....	477	45
F_3 families IS	35	.....	.....	.....	.....	.....	.....	131	2,117	25	2,308	212
Marquis IR	5	....	215	1	....	40	3	.....	.....	....	264	23
Marquis HR	16	....	326	39	12	107	9	33	83	2	627	50
Kota HS	.....	....	1	.....	.....	.....	.....	.....	15	....	16	1
Kota IS	13	....	.....	.....	.....	.....	....	3	962	....	978	81

The infection of the Kota parent fell within type 3 with the exception of one plant which had a type 1 infection. With the conditions under which the studies were made, occasional infection with a different biologic form could not be avoided. The infection of the Marquis parent fell mainly within types 1 and 2, although there were 118 plants out of 891 which showed infection of type 3.

The Marquis seed used was from a carefully selected sample and was typical of the Marquis variety. There was the possibility that these Marquis seedlings which in the greenhouse gave type 3 infections were of a different genetic nature than the greater part of the Marquis seedlings, due to mixtures or some other causes. Accordingly, seedlings which showed the type 3 and the type 1 or 2 infection in the greenhouse were planted in the field and grown to maturity. All plants appeared to be of the Marquis variety. Seeds from several plants in each group were saved separately and progeny of these plants tested in the greenhouse by inoculating with spores of Form XIX. Five out of six of the progeny of the seedling plants which had type 3 infections again gave seedlings which were placed in the 3 group, while the infection of one family was mainly in types 1 and 2. Six out of seven progeny lines which descended from seedlings having type 2 infections again had similar degrees of infection, while one family bore infection mainly of type 3. The reason, then, for the greater part of the type 3 infections obtained in the Marquis seedlings appears to be genetic differences in the Marquis material.

To determine their reaction to biologic Form XIX, 455 F_3 families were tested. Some proved homozygous in the moderately resistant and the moderately susceptible types. Other F_3 families proved to be heterozygous. The number of susceptible types is too large to be explained on the basis of a single factor difference. Forty resistant families were obtained.

Certain of the F_2 parent plants of these various F_3 families were selected and their progeny grown in the rust nursery in 1922. Some lines appeared desirable and individual plants within these lines were selected. Seedlings were grown in the greenhouse from the individually selected plants and were inoculated with Form XIX. Progenies of 3, 4, and 5 plants of three different F_3 families, which were classified as IS, or of the Kota type of infection, were grown in the greenhouse. All 12 plants bred true to the IS type of infection. One family from the IR group was tested in a similar manner. Progeny of 5 plants of this family were tested, and all showed type 2 infections, as was expected.

The reactions to Form XXVII were of a more definite nature than those to Form XIX, as will be seen in Table VI. Of the Kota plants all except 33 out of a total of 1,040 were in the immune class, while Marquis had 9 plants in type 4 and 725 in type 2 infections. The deviations obtained could all be explained on the basis of natural causes, for the frequency of such natural crossing is in the neighborhood of 2 to 4 per cent in Minnesota under nursery conditions.

A total of 462 F_3 families were tested. Of these, 76 showed either no infection or infection of the 0 or immune type, 14 were resistant, and 39 were susceptible, and there were four separate classes of heterozygous types. Immunity is clearly dominant over resistance or susceptibility as shown by the results for the heterozygous classes H1 and H4.

The segregating classes obtained when F_3 families were inoculated with Form XXVII could result from two independently inherited genetic factors for resistance and immunity each allelomorphic to a factor for susceptibility. Furthermore, if the factor for immunity was epistatic to the factor for resistance, three times as many homozygous immune families would be obtained as homozygous for either resistance or susceptibility. It is possible that a much more complex genetic condition may be the cause of the classes of segregation obtained. The clear proof that

some susceptible types may be obtained from a cross of resistant parents is of much interest.

TABLE VI.—*Distribution of the F_3 seedlings of the cross between Kota and Marquis according to types of infection caused by biologic Form XXVII*

Classes of parents and F_3 crosses.	Types of infection.						Number of plants.	Number of families.
	0	2−	2	2+	4−	4		
F_3 families R	1,159	.....	.....	.....	.....	.....	1,159	76
F_3 families IR	12	.....	55	88	.....	.....	155	14
F_3 families H1	588	2	66	113	.....	.....	769	62
F_3 families H2	707	.....	52	113	164	29	1,065	82
F_3 families H3	23	23	67	225	264	29	631	50
F_3 families H4	1,312	.....	.....	.....	305	98	1,715	139
F_3 families S	22	.....	.....	.....	387	87	496	39
Marquis H3	.....	.....	28	67	8	1	104	8
Marquis IR	9	.....	172	458	.....	.....	639	55
Kota H1	16	.....	1	.....	.....	.....	17	1
Kota H4	179	.....	.....	.....	32	1	212	15
Kota R	811	.....	.....	.....	.....	.....	811	59

Several hybrid F_3 families, whose seedling reaction to Form XXVII was known, were grown in the rust nursery in 1922, and individual plants from various progeny lines were selected. Three such lines, which in a previous greenhouse test gave reactions which placed them in the R or immune group, were again tested for their reaction to Form XXVII for the purpose of checking up the accuracy of the preceding year's study. A total of 16 plants belonging to these three families had type 0 infection and thus were in the immune class. Two lines of the susceptible group were similarly tested. One gave evidence of breeding true for susceptibility while another segregated, giving both susceptible and resistant types. If similar results were obtained, the number of families in the susceptible group should be decreased from that presented in Table VI.

The Marquis and Kota pots which were inoculated at the same time were used as controls on the purity of the biologic forms. In the hand-inoculated pots, the killed areas around the uredinia on Marquis were much less sharply defined than in the studies made the previous winter. In two pots, Marquis seedlings which were inoculated by brushing with seedlings infected with biologic Form XXVII were heavily infected with rust over the greater part of their leaf surface. The uredinia on these heavily infected seedlings were surrounded by sharply defined hypersensitive areas. Apparently environmental conditions may influence, to a rather marked degree, the reaction of seedlings to a particular biologic form.

INFECTION UNDER FIELD CONDITIONS

One of the purposes of the investigation was to determine the possibility of using the greenhouse test as a means of determining those F_3 families having the type of resistance which Kota shows under field conditions. While Kota in the field frequently is heavily infected with stem rust, the uredinia do not break out in the same manner as on susceptible varieties and are much smaller and often surrounded by a

hypersensitive area. For this reason the seed of Kota is generally well filled even under rust-epidemic conditions.

The F_3 families studied in their reaction to Form XIX and Form XXVII in the greenhouse were the progeny of F_2 plants taken at random from an F_2 population. The seed characters of these plants were examined and the plants which appeared to have desirable seed from an agronomic standpoint were selected and their progeny grown in the rust nursery in 1922. The various F_3 lines were exposed, as has been previously noted, to at least 9 biologic forms of stem rust. The epidemic was a satisfactory one. Individual plants within the different F_3 families were carefully examined and on the basis of the infection obtained the F_3 families were placed in the following classes:

R=Resistant; while there was considerable infection the plants all showed the Kota type of resistance.
NR=Semiresistant, all plants infected in a similar way. Much more resistant than Marquis, but more heavily infected than Kota.
H=Heterozygous, containing both resistant and susceptible plants.
S=Susceptible, all plants heavily infected, with large uredinia, similar to the infection shown by Marquis.

The results of the field experiment are presented in Table VII. The 9 control plots of Kota were all clearly resistant while the 20 control plots of Marquis were fully susceptible.

TABLE VII.—*Distribution of 206 F_3 families of the Marquis-Kota cross, which had not been tested previously under rust-epidemic conditions, when inoculated in the rust nursery with a mixture of 9 different rust forms, in 1922*

Parents or hybrids.	Number of families.			
	Homozygous.		Heterozygous.	Susceptible.
	Resistant.	Semi-resistant.		
F_3 awnless homozygous	3	18	3	22
F_3 bearded homozygous	15	35	16	22
F_3 heterozygous	10	33	12	17
Kota, bearded	9	.....	.....	.....
Marquis, awnless	.....	.....	.....	20

Of a total of 206 F_3 families 28 were homozygous resistant, 61 were susceptible, 86 appeared semiresistant, and 31 were clearly heterozygous. These results can not be explained on a single factor basis, because only 28 out of 206, which is a ratio of 1 to 7.36, were as resistant as Kota. It should be remembered that 9 biologic forms of rust were used to produce the field epidemic. Apparently the Kota type of resistance to all 9 forms depends on more than a single genetic factor.

In 1921 an F_2 generation of the Kota-Marquis cross was planted in the rust nursery. Several of the 9 biologic forms used in 1922 to produce the epidemic were available, although only three or four biologic forms were used to produce the 1921 epidemic. A total of 666 normal plants, which were carefully examined, consisted of 80 bearded susceptible, 233 awnless susceptible, 89 bearded apparently resistant, and 264 awnless apparently resistant. The 353 plants which appeared resistant were harvested and on examination 47 appeared to have desirable seed char-

acters. Progenies of these were grown in 1922 in the field and the rust reaction determined. Eleven of the 47 families were resistant, 14 were semiresistant, 4 were susceptible, and 12 were heterozygous. If the same relative proportion of the entire 353 resistant families had bred true to resistance there would have been 82 out of 666 F_2 plants that were homozygous in their resistance to all eight biologic forms. This is a ratio of 1 homozygous resistant to 8.1 of other types, which is very similar to the previously discussed result of the inoculation of 206 F_3 families of which 28 bred true for the Kota type of resistance.

THE GREENHOUSE TEST AS A MEANS OF ISOLATING LINES RESISTANT IN THE FIELD

One of the purposes of a study of the greenhouse reactions to Forms XIX and XXVII was to determine the possibility of using this test as a means of isolating lines which would exhibit the Kota type of resistance in the field. Fifty-two families whose reactions to Form XIX were known in the greenhouse and 48 families whose reactions to Form XXVII were known were grown in the field nursery in 1922 and classified under field conditions as resistant, near-resistant, heterozygous, and susceptible, as shown in Table VIII.

TABLE VIII.—*Distribution into classes according to reaction in the greenhouse of F_3 seedlings from F_2 plants in relation to behavior of F_3 families in the field rust nursery under artificial epidemic conditions*

Reaction under field conditions.	Greenhouse reaction—										
	To Form XIX.					To Form XXVII.					
	IR.	HR.	H.	HS.	IS.	R.	IR.	H_1.	H_2.	H_3.	H_4.	S.
R	0	1	1	0	3	1	1	0	0	0	3	0
NR	1	3	10	2	11	5	0	2	6	3	5	3
H	3	0	2	1	4	2	2	0	1	0	3	1
S	1	0	4	1	4	0	0	4	2	0	2	0

A study of the results shows that the greenhouse reactions to the biologic Forms XIX or XXVII can not be used to determine which families will exhibit the Kota type of resistance to several biologic forms in the field. Thus of the five families which gave the Marquis type of reaction to Form XIX in the greenhouse, namely, the IR group, none bred true for resistance under field conditions. Similarly, of the 22 families which gave the Kota type of reaction in the greenhouse and which were placed in the IS group, only 3 proved resistant in the field. The reaction of Kota to Form XXVII was of the immune or R type. Eight hybrid families of this type were grown in the field and only one proved as resistant as Kota. These results are further evidence which proves that the resistance of Kota to several biologic forms of stem rust is due to more than a single genetic factor.

QUESTION AS TO WHETHER THE RESISTANCE TO FORM XIX AND FORM XXVII CAN BE COMBINED IN A SINGLE VARIETY

In order to build up wheats resistant to all biologic forms, it is necessary to combine in a single variety the resistance exhibited by different wheat varieties to particular biologic forms. The reactions of F_3 families to Forms XIX and XXVII are correlated in Table IX.

TABLE IX.—*Distribution of F_3 families classified according to reaction to form XIX in relation to reaction to Form XXVII*

Reaction to Form XXVII.	Reaction to Form XIX.					
	IR.	HR.	H.	HS.	IS.	Total.
R	3	9	13	5	44	74
IR	4	2	1	0	1	8
H_1	6	6	12	4	14	42
H_2	7	11	13	7	29	67
H_3	1	5	15	3	13	37
H_4	3	9	27	15	59	113
S	1	2	8	1	19	31
Total	25	44	89	35	179	372

Three out of a total of 372 families proved immune from Form XXVII and were resistant to Form XIX, and 4 families were resistant to both biologic Forms XIX and XXVII. It is possible, therefore, to combine in a single variety the resistance of the Marquis parent to Form XIX, and the immunity of the Kota parent from Form XXVII. These facts give further hope for the belief that resistance to different biologic forms exhibited by different wheat varieties is due to independently inherited Mendelian factors or that the linkage of these factors is so slight that crossovers frequently occur. From the genetic standpoint the facts so far learned give reason for the belief that a wheat variety can be produced which will be resistant to all biologic forms of stem rust.

CORRELATION BETWEEN MORPHOLOGIC AND SEED CHARACTERS AND RUST RESISTANCE

The F_2 plants which were the parents of the F_3 families tested under field conditions were classified as normal awnless or bearded and dwarf awnless or dwarf bearded. For the purpose of determining whether there was any marked correlation between these characters and reaction to rust, the reactions of these separate morphologic groups to Form XIX and Form XXVII have been arranged in correlation, as shown in Tables X and XI.

TABLE X.—*Distribution of F_3 normal awnless (A) and bearded (B) plants and dwarf awnless (DA) and bearded (DB) plants, classified according to their reaction to Form XIX in relation to spike characteristics of F_2 plants*

F_3 plant type.	F_3 reaction to Form XIX.				
	IR.	HR.	H.	HS.	IS.
A	28	35	78	28	142
B	10	12	23	11	46
DA	1	0	12	1	19
DB	0	2	3	1	5
Total	39	49	116	41	212

TABLE XI.—*Distribution of F_3 normal awnless (A) and bearded (B) plants and dwarf awnless (DA) and bearded (DB) plants, classified according to their reaction to Form XXVII in relation to spike characteristics of F_2 plants*

F_3 plant type.	F_3 reaction to Form XXVII.						
	R.	IR.	H_1.	H_2.	H_3.	H_4.	S.
A	62	11	47	60	32	101	27
B	28	3	11	20	11	30	8
DA	2	0	1	1	2	5	2
DB	1	0	1	2	2	2	2
Total	93	14	60	83	47	138	39

Three times as many awnless families as bearded were expected. The fact that there were about three times as many awnless as bearded families in each of the groups classified according to reaction to both biologic forms seems very convincing proof that the factors which govern susceptibility or resistance to either Form XIX or Form XXVII are inherited separately from the factors which govern the presence or absence of awn development.

To Form XXVII the reaction of the F_3 families from the dwarf types seems very similar to that obtained from normal plants. For Form XIX, however, the percentage of dwarf plants which were as resistant as Marquis and which were placed in the IR group is very small and certainly much less than the percentage of similar families from the normal plants.

In a somewhat similar manner the relation between average length of seed of the F_2 plants and the reaction of these plants to Forms XIX and XXVII was determined, as shown in Tables XII and XIII.

TABLE XII.—*Distribution of F_2 plants classified according to their reaction to Form XIX in relation to length of seed*

Classes by reaction to Form XIX.	Classes for length of seed (in millimeters).						Total.	Mean.
	4.8	5.1	5.4	5.7	6.0	6.3		
IR	.....	.....	5	17	9	3	34	5.79±0.03
HR	1	3	5	21	18	1	49	5.74±0.03
H	3	7	18	50	30	2	110	5.69±0.02
HS	1	.....	6	18	15	.....	40	5.75±0.03
IS	2	10	33	81	67	9	202	5.74±0.01

TABLE XIII.—*Distribution of F_2 plants classified according to their reaction to Form XXVII in relation to length of seed*

Classes by reaction to Form XXVII.	Classes for length of seed (in millimeters).						Total.	Mean.
	4.8	5.1	5.4	5.7	6.0	6.3		
R	.....	2	15	46	24	6	93	5.75±0.02
IR	.....	.....	4	5	3	2	14	5.77±0.06
H_1	2	3	8	25	20	2	60	5.72±0.03
H_2	.....	2	17	32	28	3	82	5.75±0.02
H_3	1	4	11	19	10	1	46	5.63±0.03
H_4	1	3	17	60	52	2	135	5.77±0.01
S	.....	4	5	15	13	1	38	5.72±0.03

The ranges of variability for seed length are somewhat greater, as would be expected, for the reaction groups which contain the largest number of families. The differences obtained in the means are not sufficiently great to indicate any marked linkage between the factors of inheritance which govern seed length and those which govern the manner of reaction to the forms of stem rust used in the study.

SUMMARY

(1) Kota, which is resistant to stem rust in the field but which has weak straw when grown under humid conditions, was crossed with Marquis, which is susceptible to stem rust but which possesses other desirable economic characters.

(2) A survey of available biologic forms led to the selection of Form XIX, to which Marquis was resistant and Kota moderately susceptible, and Form XXVII, from which Kota was immune and to which Marquis was resistant. These two forms of stem rust were chosen for the study with the hope of finding a greenhouse test which could be used as a means of isolating F_3 families which would prove as resistant as Kota under field conditions.

(3) Out of a total of 787 F_2 plants of the Kota-Marquis cross which were grown under field conditions, 666 were of normal type and 121 were dwarfs. The results were explained on the basis that one of the parents contained a factor for dwarf habit and another factor which prevented the production of dwarfs. The other parent apparently lacked both factors.

(4) The seeds of the F_2 bearded plants were 0.1 mm. longer on an average than the seeds from the awnless plants. The seeds of the awnless plants had slightly harder texture but were slightly inferior in plumpness to those from the bearded plants. Apparently the beard of wheat, under the conditions of the experiments, leads to the production of somewhat better developed seed.

(5) Marquis, in the greenhouse, was more resistant than Kota to Form XIX. Varying environmental conditions, however, resulted in the production of a type of infection on Marquis which approached that obtained normally with Kota. The reactions of Kota to Form XIX under the same conditions did not show similar variations in the types of infection. F_2 hybrid families were obtained which reacted in a manner similar to Marquis and Kota, while other families were obtained which were clearly heterozygous. The results could not be explained on the basis of a single genetic factor.

(6) Kota proved immune from Form XXVII in the greenhouse, while Marquis proved resistant. F_2 families were obtained which were as homozygous for immunity and resistance as Kota and Marquis, respectively, while others were obtained which were entirely homozygous for susceptibility. Four types of heterozygous families were obtained. Immunity appeared to be dominant to both resistance and susceptibility. The number of seedlings inoculated in each family available for the study was too small to make the experiment absolutely conclusive. The results could be quite satisfactorily explained on the basis of two independently inherited factors for immunity and resistance contained in the Kota and Marquis parents, respectively, each factor being allelomorphic and dominant to a factor for susceptibility.

(7) Nine biologic forms of stem rust which were collected in the North-Central and Northwestern States were used for producing a field epidemic. Kota proved rather highly resistant under field conditions while Marquis was very susceptible. In one group of 206 F_3 families there were 28 which were as resistant as Kota. This is a ratio of 1 to 7.36. In another test there was a ratio of 1 resistant F_3 family to 8.1 susceptible and heterozygous.

(8) The reaction of these F_3 families to Forms XIX and XXVII in the greenhouse was correlated with the behavior of such F_3 families under field conditions when an epidemic of stem rust was induced by infection with nine biologic forms of stem rust to which Kota proved resistant in the field. The study of F_3 seedlings in the greenhouse in their reaction to Forms XIX and XXVII was not satisfactory as a means of isolating F_3 lines which would exhibit the Kota type of resistance under field conditions.

(9) The resistance of Marquis to Form XIX and the immunity of Kota from Form XXVII were combined in 3 F_3 families out of a total of 372 families studied. This is further evidence in support of the belief that there are several genetic factors which determine the differential reactions of Marquis and Kota to the biologic forms in question. That the resistance of one parent to Form XIX and the immunity of the other parent from Form XXVII can be combined in a single hybrid family is added reason for the hope that resistance to all biologic forms can be obtained eventually.

(10) There was no close linkage in inheritance of the factors which govern the presence or absence of the awns or of seed length with those which determine the resistance or susceptibility to either biologic form used in the greenhouse study.

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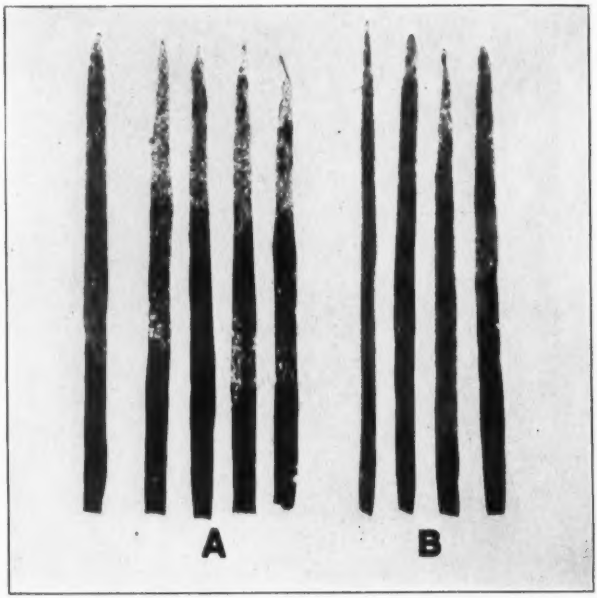
PLATE 1

Comparative lodging of Kota and Marquis. At left, Kota, badly lodged; at right, Marquis, fully erect. Photograph taken at University Farm, St. Paul, Minn., 1922.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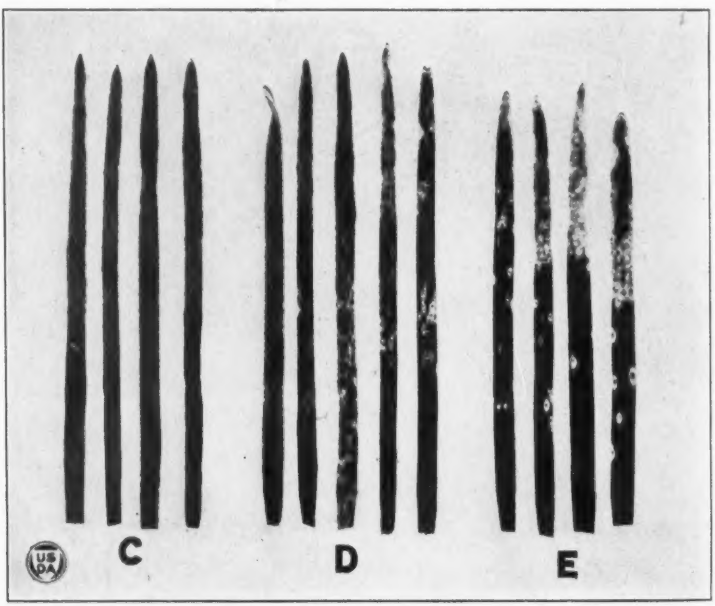
C
D
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PLATE 2

Reaction of Marquis and Kota and of F_3 families of the Marquis × Kota cross to biologic Form XIX.

A.—Reaction of Marquis of types 1 and 2 except the leaf at the extreme left, which proved to have a mixture of infection types.

B.—Reaction of Kota of type 3.

C.—F_3 family of the immune-susceptible group.

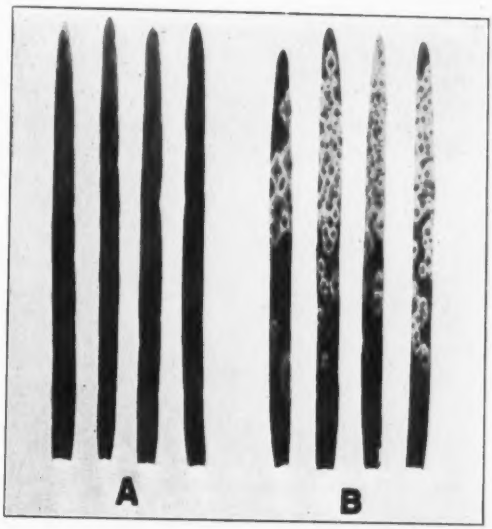
D.—F_3 heterozygous family.

E.—F_3 family of the immune-resistant group.

PLATE 3

Reaction of Marquis and Kota and of F_3 families of the Marquis×Kota cross to biologic Form XXVII.

A.—Kota, showing immunity represented by infection of type o.
B.—Marquis, showing resistance which classifies it in the immune-resistant group.
C.—Immune family.
D.—Resistant family.
E.—Susceptible family.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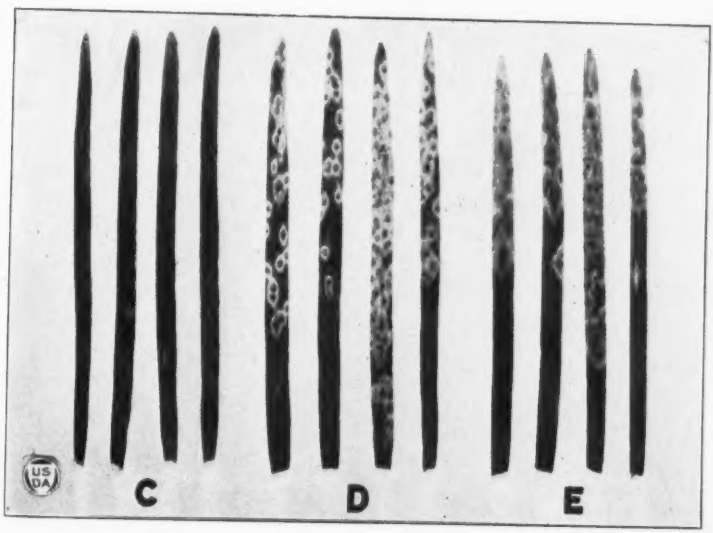
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BIOLOGIC FORMS OF PUCCINIA GRAMINIS ON VARIETIES OF AVENA SPP.[1]

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Puccinia graminis tritici Erikss. and Henn., which originally was considered as a single biologic form, has been shown by Stakman and Piemeisel (*11*),[3] Levine and Stakman (*5*), Melchers and Parker (*7*), Stakman, Levine, and Leach (*10*), and Stakman and Levine (*9*) actually to consist of many forms which differ in their parasitic action on certain varieties of *Triticum* spp. Hoerner (*4*) showed that there is a similar specialization of *Puccinia coronata* Cda. on varieties of *Avena* spp. and Mains and Jackson (*6*) found two biologic forms of *P. triticina* Erikss. on varieties of wheat. It seemed quite likely, therefore, that *P. graminis avenae* Erikss. and Henn. might also consist of several biologic forms. Experiments, therefore, were begun in the fall of 1918 to ascertain whether this was true.

It is obvious that one of the most direct methods for ascertaining whether there are biologic forms of rust, with different parasitic capabilities on varieties of cereals, is to find forms of rust capable of infecting varieties ordinarily practically immune from described rust forms, or vice versa. As a result of preliminary studies on *Puccinia graminis avenae* it was found that Victory (both C. I. No. 1145 [4] and Minn. 514), Improved Ligowa (Minn. 281), Minota (Minn. 512), and many other varieties of oats were extremely susceptible to the rust strains [5] used, while White Tartar (White Russian) (Minn. 339 and two pure-line selections made by the section of plant pathology at the Iowa Experiment Station and designated as Rust Nursery Row No. 101½ and 102½), was quite resistant. The writers, therefore, began collecting uredinial material of *P. graminis* Pers. on oats from as many different localities as possible, in this and other countries.

Since the fall of 1918, more than 100 collections of *P. graminis* on oats have been obtained from 21 States of the United States, from two Provinces in Canada, and from one State in Mexico. Seventy-six of the collections have been cultured in the greenhouse of the section of plant pathology at University Farm, St. Paul, Minn. Many of these were used for inoculating seedlings of about 70 different varieties and selections

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[2] The writers are indebted for rust material to Prof. W. P. Fraser, pathologist in charge of Cereal Disease Investigations, Dominion Laboratory of Plant Pathology, in cooperation with the University of Saskatchewan, Saskatoon, Sask, Canada; to Prof. Wallace Butler, of San Antonio, Tex.; to Mr. G. F. Puttick, of the Department of Agriculture of the Union of South Africa; and to Mr. Fred Griffee, assistant plant breeder, Department of Agriculture, University of Minnesota. They also are indebted to other Federal and State pathologists for collections of rust.

Dr. H. K. Hayes, plant breeder, Department of Agriculture, University of Minnesota and collaborator of the Office of Cereal Investigations, Bureau of Plant Industry, U. S. Department of Agriculture, made valuable suggestions for which the authors wish to express their appreciation.

[3] Reference is made by number (italic) to "Literature cited," p. 1017–1018.

[4] C. I.=Cereal investigations accession number.

[5] The term "strain" is used only to designate a culture of rust from a single collection.

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of oats, belonging to 6 different species of the genus Avena, namely: *A. brevis* Roth., *A. nuda* L., *A. sativa* L., *A. orientalis* L., *A. sterilis* L., and *A. strigosa* Schreb.

It was found that definite preliminary results were likely to be obtained by using only three varieties of oats, viz: Victory, White Tartar (White Russian) and Monarch Selection (of Etheridge). All collections were run to Victory and White Tartar (White Russian). Victory was completely susceptible to all of the strains tried, White Tartar was very highly resistant to most of them, while Monarch Selection, which was inoculated only with the collections made in 1921 and 1922, was extremely susceptible to some of these collections and practically immune from others. Attempts then were made to find forms of stem rust which would infect the White Tartar variety heavily. This was achieved with rust material procured from Europe and Africa.

Victory was completely susceptible to, White Tartar very resistant to, and Monarch Selection (of Etheridge) practically immune from, a form collected by Mr. Fred Griffee at St. Paul, Minn. This is designated hereafter as Form I.

A form of rust collected by Prof. Wallace Butler at San Marcos, Tex., infected Victory normally, developed only lightly on White Tartar, but produced large, vigorous uredinia on Monarch Selection. These results were confirmed by repeated inoculations, and clearly indicated the existence of a second biologic form of *Puccinia graminis avenae*, as it was capable of infecting Monarch Selection normally, which the form collected by Mr. Griffee could not infect. This is designated as Form II.

A rust strain sent by Mr. G. F. Puttick from Potchefstroom, Union of South Africa, infected Victory normally, White Tartar moderately, and Monarch Selection normally. This undoubtedly was a third form and is hereafter called Form III.

Finally, Stakman found a form at Upsala, Sweden, which infected White Tartar just as heavily as it did Victory and Monarch Selection. This, then, was a fourth form,[6] designated as Form IV.

Table I summarizes the action of these four forms on the varieties of oats which served as the differential hosts.

It is quite evident from Table I that Form I (Pl. 2, A) infects Victory normally, but develops only lightly on White Tartar (White Russian) and exceedingly lightly on Monarch Selection. Form II (Pl. 2, B) infects Victory and Monarch Selection heavily, but attacks White Tartar only lightly. Form III (Pl. 3, A), like Form II, attacks Victory and Monarch Selection very heavily and in addition attacks White Tartar moderately. Form IV (Pl. 3, B) infects all three varieties very heavily. Repeated inoculations have been made and the same results have been obtained consistently.

Two different forms were isolated from a single collection several times. In some cases the X type of infection described for wheat rust (*9, p. 5*) also developed on oats. Several strains produced this X type of infection on Monarch Selection, thus indicating the probable existence of a fifth biologic form which infects Victory very heavily, White Tartar only lightly and Monarch Selection heterogeneously (Pl. 4). There are now known, therefore, at least four, and probably five, biologic forms of *Puccinia graminis avenae* which produce different reactions on certain varieties of *Avena* spp.

[6] Proper disposition is being made of the spore material of the South African and Swedish forms, as they have not yet been found in the United States.

TABLE I.—*Results of inoculating three differential varieties of Avena spp., with four biologic forms of Puccinia graminis avenae*

Biologic Form No.	Place of collection.	Summary of inoculation results.					
		Victory (C. I. No. 1145).		White Tartar (Minn. 339).		Monarch Selection (of Etheridge).	
		Number of trials[a]	Character of infection.	Number of trials.	Character of infection.	Number of trials.	Character of infection.
I	St. Paul, Minn.	19	Normal, heavy infection. Uredinia large, numerous and coalescing.	8	Infection light. Uredinia usually small and scattered. Hypersensitiveness (sharp chlorosis to definite necrosis) ordinarily present.	6	Infection exceedingly light. Uredinia, when present, minute, scattered and surrounded by very sharply defined necrotic areas. Distinct hypersensitive flecks also occur quite frequently.
II	San Marcos, Tex.	16	.....do.....	8	.....do.....	6	Normal, heavy infection. Uredinia large, coalescent and numerous.
III	Potchefstroom, South Africa.	7	.....do.....	5	Infection moderate. Uredinia medium in size and only slightly confluent. True hypersentitiveness absent, but light chlorotic areas usually present.	3	Do.
IV	Upsala, Sweden.	2	.....do.....	2	Normal, heavy infection. Uredinia large, many, and confluent.	2	Do.

[a] From 10 to 15 plants were inoculated in each trial.

These newly discovered biologic forms are quite as distinct as are those of *P. graminis* on wheat varieties (*12*). The inoculations were made on seedlings in the greenhouse, but, as a result of previous work, it is safe to conclude that the reaction of seedlings to rust forms is a fairly accurate index of the reaction of older plants. It usually is more difficult to obtain infection on older plants than it is on seedlings, but this apparently is due very largely to the fact that the film of water necessary for spore germination and entrance of germ tubes through the stomata, is not easily maintained on old plants. However, when this difficulty is overcome, by atomizing the plants several times a day, infection occurs normally.

The new forms apparently are constant. Repeated inoculations and cross inoculations have been made with most of them and the results have been consistent. There are variations, of course, in the intensity of rust development when the environmental conditions are unfavorable for the development of host or parasite or both. When light intensity is low, and when the temperature is either too high or too low, the rust develops subnormally. If the host plants are weakened by poor growing conditions, by mildew, root rots, or other factors, the rust usually does not develop well. This variation, however, does not indicate any change in the genotypic constitution of the rust forms, but is only the temporary result of environment. In order to draw accurate conclusions regarding the parasitic behavior of biologic forms it is necessary to grow the host plants and the rust fungus within the range of environmental conditions in

which they will develop normally. Optimum conditions are not essential but extremely unfavorable conditions should be avoided.

It is important to determine whether the urediniospores of these new forms are really of the avenae type, as *P. graminis phleipratensis* (Eriksson. and Henn.) Stak. and Piem. develops moderately well from artificial inoculation on some varieties of oats and may possibly occur on some in the field. *P. graminis agrostis* Eriksson. also can attack some oat varieties weakly. These forms, however, can be distinguished easily from *P. graminis avenae* by the morphological characters of the urediniospores. The urediniospore characters of the new forms clearly are those of *P. graminis avenae* (*8*). However, it is not yet known whether these individual forms differ appreciably from each other morphologically.

The geographical distribution of these biologic forms of *P. graminis avenae* is not yet known definitely. The sources of 23 collections, made during 1921 and 1922, are given in Table II. Form I has been collected from Saskatchewan to Mexico, in the central part of the continent, and probably is widely distributed.

TABLE II.—*Distribution of biologic forms of stem rust of oats collected during 1921 and 1922*

Biologic Form.	Place of collection.	Original host.
II	Saskatoon, Sask., Canada	Avena sativa L.
[a] V	Lloydminster, Sask., Canada	Do.
[a] V	Weyburn, Sask., Canada	Do.
I, II	Zelma, Sask., Canada	Do.
II	Presque Isle, Me	Do.
I, II	Litchfield, Minn	Do.
I, II	Rosetown, Minn	Do.
I	St. Paul, Minn	Do.
II	Redfield, S. Dak	Do.
II	Huron, S. Dak	Do.
[a] V	Colmar, Iowa	Do.
I, II	Lafayette, Ind	Poa trivialis L.
I	Huntley, Ill	Avena sativa.
II	Chillicothe, Mo	Do.
II	Norman, Okla	Dactylis glomerata L.
II	Lawton, Okla	Avena sativa.
II	San Marcos, Tex	Do.
I, II	Boerne, Tex	Do.
I, II	San Antonio, Tex	Do.
I, II	Robards, Tex	Do.
I, II	Saltillo, Mexico	Do.
IV	Upsala, Sweden	Do.
II, III	Potchefstroom, South Africa	Do.

[a] If not a mechanical mixture of I and II.

Form II seems to be even more abundantly distributed than Form I in the central part of North America, from Saskatchewan to Mexico, and has been found also in Maine, as well as in South Africa.

Forms III and IV have not yet been found in North America. It seems improbable that they are abundant in the northern Mississippi Valley, because the White Tartar (White Russian) oat is very resistant in the field. Furthermore, Garber (*3*) produced Victory × White Russian hybrids which were resistant to stem rust, at least in Minnesota. As these

hybrid strains combine the desirable characters of Victory with the rust resistance of White Tartar, it is to be hoped that Forms III and IV of *P. graminis avenae* do not exist in the United States. If they do not, the problem of developing good varieties of oats resistant to stem rust seems to be well on the way to solution.

The provisional Form V was collected in two localities in Saskatchewan, Canada, and in one in Iowa. It may be a mechanical mixture of Forms I and II. Inoculation experiments to determine this fact are in progress.

It is especially interesting to note that the Swedish form of rust is more virulent than any of those so far found in the United States, because Eriksson (*2*) long ago called attention to the fact that *P. graminis avenae* was exceptionally virulent in Sweden. The senior author was also impressed with this fact during a recent visit to Sweden. Several grasses, which are moderately susceptible to the avenae form of rust in the United States, but which seldom rust heavily in the field, were very generally and severely infected in Sweden. The fact that there are tremendous numbers of barberry bushes in some oat-growing regions of Sweden, and the additional fact that oats are so commonly grown might account for the abundance of the rust. In addition, however, Eriksson evidently was correct in assuming that the stem rust of oats was especially virulent in Sweden. This emphasizes still more the fact that the specialization of *P. graminis avenae* may be different in different countries. The present results indicate that the forms may also differ even in various regions of the same country. On the other hand more than one biologic form may occur in the same locality or even on the same plant.

While the virulent Swedish form of stem rust of oats may possibly exist in the United States, fortunately it does not seem to be either abundant or widespread in those Northern States in which White Tartar is commonly grown. The work of Durrell and Parker (*1*) indicates also that White Tartar is quite generally resistant to stem rust.

The practical significance of the existence of several biologic forms of *Puccinia graminis* on oats in the United States will depend on the number and the virulence of forms which may be found in the future. This problem is now being thoroughly investigated.

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PLATE 1

Classes of host reaction: Resistant (R), susceptible (S), and intermediate (X), as indicated by different types of infection (0, 1, 2, 3, 4, and X) produced by biologic forms of stem rust on different varieties of oats.

Class R (resistant), including 3 subclasses corresponding to infection types 0, 1, and 2.

A.—Practically immune (0). No uredinia are developed, but sharply defined hypersensitive flecks are usually present.

B.—Extremely resistant (1). Infection very light; uredinia minute and scattered and surrounded by very sharply defined necrotic areas.

C.—Moderately resistant (2). Infection light; uredinia usually small and scattered; hypersensitive areas varying from sharply defined necrosis to pronounced chlorosis.

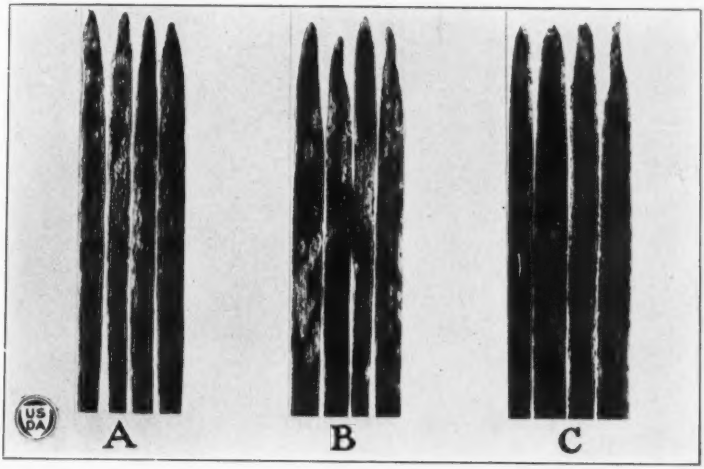
Class S (susceptible), including two subclasses corresponding to infection types 3 and 4.

D.—Comparatively susceptible (3). Infection moderate; uredinia midsized with a tendency to coalesce; true hypersensitiveness absent, but light chlorotic areas usually present.

E.—Completely susceptible (4). Infection normal and heavy; uredinia large, numerous and confluent; hypersensitiveness entirely absent, but chlorosis may be present when cultural conditions are unfavorable.

Class X (intermediate), representing the heterogeneous (X) type of infection. (No subdivisions of this class are recognized.)

F.—Uredinia very variable, apparently including all types and quantities of infection, often of the same blade; no mechanical separation seems to be possible, as, on reinoculation, spores from small uredinia may produce large ones, and vice versa. In general, the infection is ill-defined.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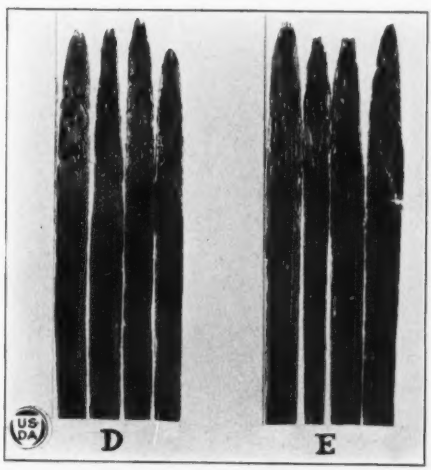
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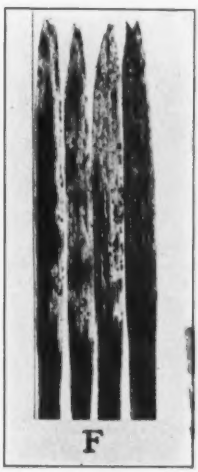
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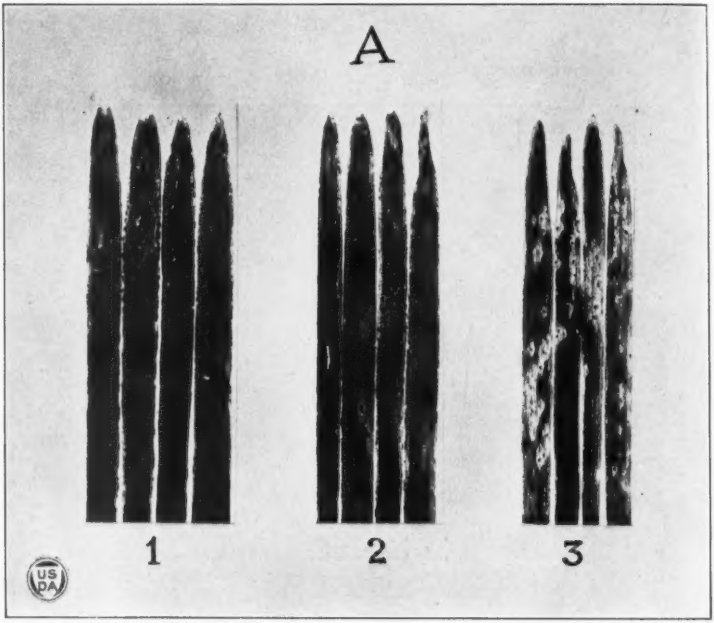
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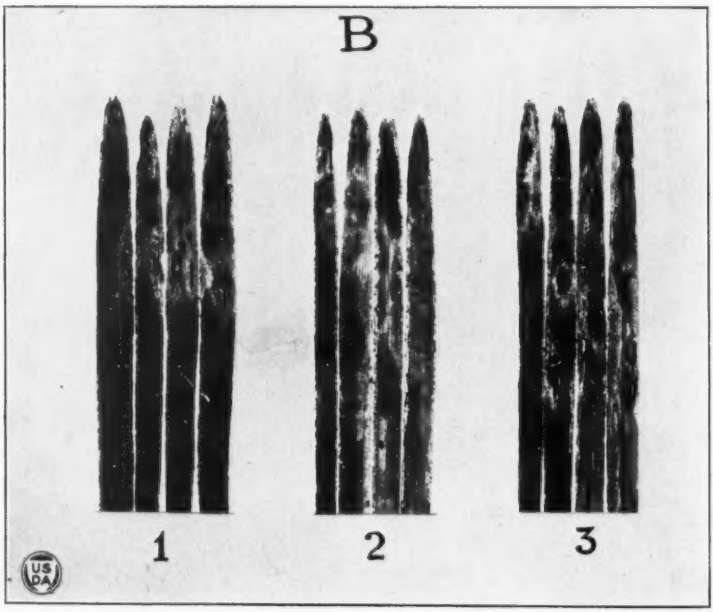
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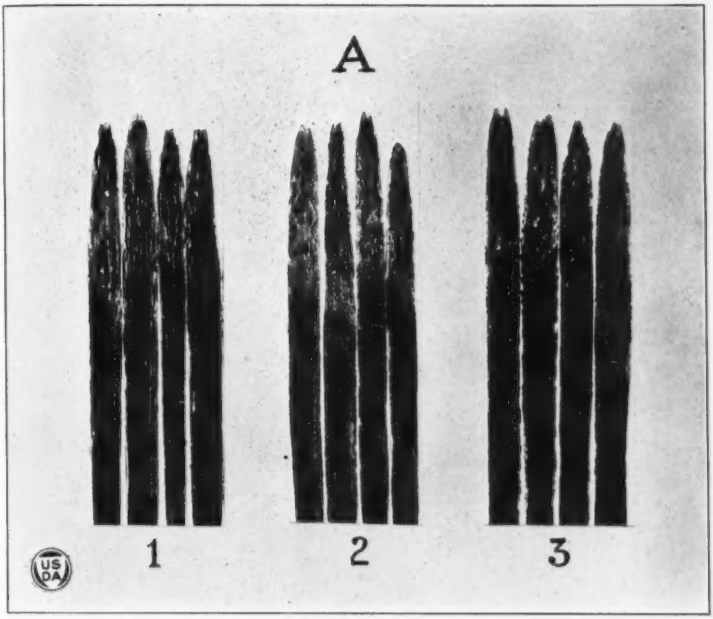
PLATE 9

A.—*Puccinia graminis avenae* (Form I): (1) Victory, very susceptible; (2) White Tartar, quite resistant; (3) Monarch Selection, practically immune.
B.—*Puccinia graminis avenae* (Form II): (1) Victory, very susceptible; (2) White Tartar, quite resistant; (3) Monarch Selection, highly susceptible.

PLATE 3

A.—*Puccinia graminis avenae* (Form III): (1) Victory, completely susceptible; (2) White Tartar, moderately susceptible; (3) Monarch Selection, very susceptible.
B.—*Puccinia graminis avenae* (Form IV): (1) Victory, completely susceptible; (2) White Tartar, highly susceptible; (3) Monarch Selection, very susceptible.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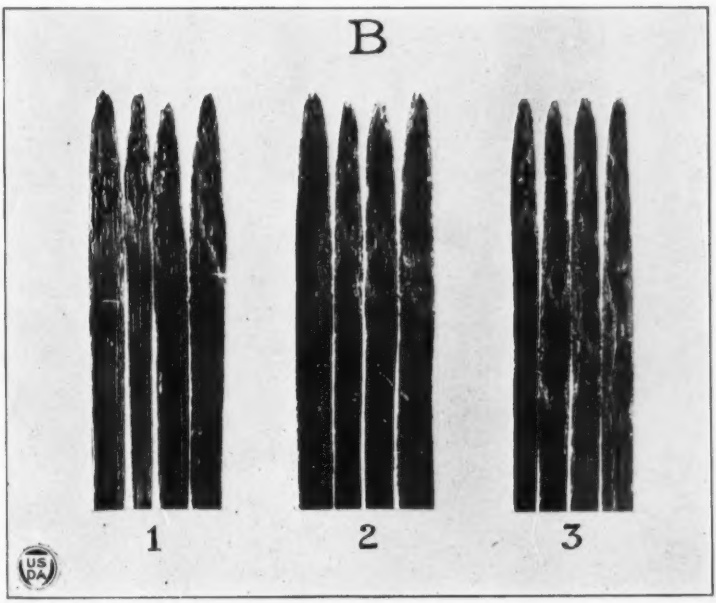
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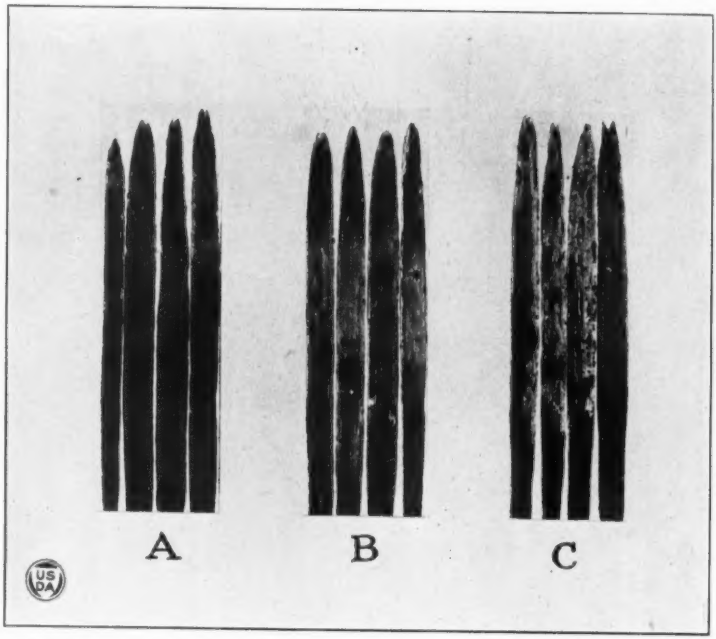
A
B
C

PLATE 4

Puccinia graminis avenae (Form V)

A.—Victory, susceptible.
B.—White Tartar, resistant.
C.—Monarch selection, intermediate.

DISEASE RESISTANCE TO ONION SMUDGE[1]

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INTRODUCTION

In an earlier paper on onion smudge (*24*)[3] a description of the symptoms and seasonal development of the disease, and of the morphology and life history of the causal organism is given. In connection with the above studies it was found in confirmation of the earlier observations of Berkeley (*1*), Thaxter (*18, p. 163–165*), Halsted (*8*), and Selby (*16, p. 364–366, 414*), that all white varieties of onion observed are highly susceptible to smudge while varieties with colored bulbs—yellow and red—show a high degree of resistance. Further study as to the nature of this resistant quality has shown that there are at least two substances or groups of substances within onion tissue which have a marked inhibitive effect upon the growth of the causal fungus, *Colletotrichum circinans* (Berk.) Voglino. One of these is the volatile oil,[4] and the other is a group of one or more compounds closely associated or identical with the flavone and anthocyan pigments of the scales. The present paper contains the results of investigations upon this subject.

OCCURRENCE OF VARIETAL RESISTANCE AND SUSCEPTIBILITY TO ONION SMUDGE

Observations on the relative susceptibility or resistance of onion varieties to smudge in field and storage have been continued since 1914, chiefly in Wisconsin and Illinois but incidentally in a number of other onion-growing sections in the United States. In 1922, the opportunity was given to secure field data from certain onion centers in Europe and the Canary Islands. In all cases there, as in this country, where colored and white varieties were grown simultaneously under conditions favorable for the development of smudge, white bulbs were uniformly infected while colored bulbs were completely free or infected to only a slight degree.

It should be pointed out in this connection that the pigment in colored varieties of onion appears in the outer bulb leaves or scales when the plants are about half grown, or earlier. Several of the first leaves of the plant acquire no basal thickness and the first of these slough off, without the production of much pigment, during the plant's early growth. With age the coloring becomes more intense until a short time before maturity,

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[2] The writer is indebted to Prof. L. R. Jones of the University of Wisconsin and to others in both the university and the Department of Agriculture for valuable suggestions and criticisms during the progress of the investigation.

[3] Reference is made by number (italic) to "Literature cited," p. 1038–1039.

[4] Throughout this paper the term "volatile oil" is used to designate the volatile substances which ordinarily arise from onion tissue, some of which, at least, are responsible for the characteristic onion odor.

when the yellow or red pigment, depending on the variety, is fully developed. At this time the plants have one to three thin, papery outer scales which are intensely colored. These cover the succulent scales and extend up over the neck of the bulb, where they frequently connect with their respective green portions of the tubular leaves or "top," although the latter may have disappeared by this time. Following these outer scales upward from the base, we find that the pigment ceases very abruptly above the bulb, leaving the neck sheathed largely by uncolored tissue (Pl. 1). Toward the end of the growing season there is among colored varieties a tendency toward some pigment reduction in the outer scales, especially in the portions above the soil line. This becomes more noticeable when bulbs are allowed to remain in the ground for a prolonged period after they are mature and ready for harvest.

When the disease appears early, several weeks before harvest, a few fruiting bodies of the fungus are occasionally found on the dead outer scales of colored varieties. These scales, usually only slightly pigmented, slough off before harvest. Practically all of the subsequent infection on colored varieties is confined to the outer sheath at or just above the neck of the bulb. This tissue, like the lower portions of the outer scale, is practically dead previous to harvest, but little or no pigment is present. This condition is illustrated in Plate 1, which shows one set each of red, yellow, and white varieties, all taken from the same field a few days before harvest. It is to be noted that the disease has become general on the bulb of the white variety, while in the yellow and red varieties it is confined to the uncolored sheath of the neck. In onion-set fields, this condition of infection at the neck very often becomes general on colored varieties if there is sufficient rainfall and high relative humidity to bring about dissemination of the fungus and infection. The intensely colored portions of the outer scales rarely show signs of the disease. The infection at the neck is largely sloughed off at harvest, leaving little or no sign of the disease in storage. On white varieties, as stated above, the fungus usually covers the entire bulb and continues to invade the fleshy scales, causing a gradual shrinkage.

INOCULATION EXPERIMENTS WITH BULBS

The field observations reported above have been confirmed by plot experiments in which the plants were grown on well-infested soil or were inoculated three to four weeks before harvest with spore suspensions of the causal organism. In these experiments a number of varieties of each color were included. Table I gives a list of the varieties used and the dates of the trials. These trials confirm the general field data in that the white varieties all appeared to be highly susceptible, while the colored varieties were highly resistant to the disease. In the case of the latter group, where infection did occur it was present in small amounts and usually at the neck of the bulbs where the pigment was less intense. Occasionally the outer pigmented scales bore some fungus stromata, but usually the latter were associated with a lessening of pigment due to one cause or another. In other instances, invasion of the fleshy scales was noted where they were exposed to the soil, due either to splitting or to partial disintegration of outer scales when the bulbs were not harvested promptly. This observation is in accord with the following experimental evidence which shows that when the fungus is applied directly to the fleshy colored scale, invasion readily takes place.

TABLE I.—*List of onion varieties tested as to their susceptibility or resistance to smudge*

Color.	1916	1917	1920	1921
White.	White Globe. White Portugal. Queen. Mammoth Silver King. Extra Early Barletta. Giant White Italian Tripoli.	White Globe. White Portugal. Queen. Giant White Italian Tripoli.	White Globe.	Crystal Wax Bermuda. White Australian. White Portugal. Early White Queen.
Yellow or brown.	Southport Yellow Globe. Michigan Yellow Globe. Yellow Globe Danvers. Yellow Danvers. Yellow Strassburg. Prizetaker. Early Neapolitan Marzola. Australian Brown.	Southport Yellow Globe. Michigan Yellow Globe. Yellow Globe Danvers. Yellow Strassburg.	Yellow Globe.	Ailsa Craig. Gigantic Gibraltar. Australian Brown.
Red.	Extra Early Red. Large Red Wethersfield. Southport Red Globe.	Extra Early Red. Large Red Wethersfield.	Red Globe.	Extra Early Red. Red Bermuda.

The inoculation studies were continued in moist chambers, where drops of spore suspension were placed directly upon marked areas of the surface of the scales. From certain of the bulbs the dry, outer scales were removed and the inoculum placed directly upon the succulent scale, while in others the natural conditions were simulated by placing the drops upon the dry scale. In no case did any infection whatever occur upon the dry outer scales of colored bulbs; and where the spores were examined in the drops of inoculum no normal germination had occurred. In nearly all instances, however, inoculations in the same or similar moist chambers upon the succulent scales of bulbs from the same original source did take effect. The infected area was characterized first by a very rapid disappearance of the pigment, often within two days after the inoculations were made, followed by a gradual collapse of the epidermal and underlying cells (Pl. 2, A). From all appearances the fungus invaded the so-called resistant varieties as aggressively as it did the susceptible ones when the dry scales were removed. The outer scales, therefore, appear to serve as the barrier and to be responsible for the resistant property of the colored varieties.

The rapid destruction of the coloring matter in succulent scales at first was very surprising in view of the marked toxicity of water extract of pigmented scales, which will be described later. In fact, it necessitated a further study of fungus invasion of the colored succulent scales. This was done by cutting tangential razor sections from the surface of the scale directly beneath the drop of inoculum so as to contain the undisturbed epidermis with a few layers of the underlying cells. These sections were then examined in water mounts under the microscope. By this method the progress of the fungus could be watched from the instant of spore germination throughout the disappearance of the pigment. Scales of a red variety were used because it was much easier to follow the latter phenomenon in them than in those of the yellow varieties. It was found that the process of invasion was exactly similar to that already described for white onion bulbs (*24*). After the formation of the appressorium and of the penetration tube, the mycelium developed first between the cuticle and sub-epidermal wall. Many cells were to be

found in which penetration of the cuticle had occurred with no apparent reduction of pigment. However, before the hyphae had developed to any great extent there was evidence of pigment reduction, which proceeded quite rapidly. From the evidence so far obtained it appears that the pigment was destroyed while the mycelium was developing in the outer wall and before actual penetration of the cell occurred. If this be true, the hyphae do not actually come into contact with the pigment as such; hence even if the latter were toxic, it might have no effect upon the fungus. The destruction of the pigment is due either to a substance secreted by the hyphae and capable of diffusing in advance of them or to independent chemical changes in the host cell brought about by the disturbances resulting from fungus invasion of the outer wall.

Of interest in this connection is the common observation made during these experiments—namely, as the lesion developed on the succulent red scales, pigment of normal intensity appeared in the lumina of several layers of the uninvaded parenchyma cells below and around the lesion. In normal, healthy scales these cells never contain pigment, the latter being confined to the cells of the outer epidermis. The significance of the appearance of pigment under these adverse conditions can not be entirely explained, but it seems to show very conclusively that the fungus infection at a given point on the scale not only affects the invaded cells but also influences either directly or indirectly the metabolism of uninvaded cells for some distance around the lesion. The stimulation of anthocyan production by fungus invasion is a matter of common observation in nature (*25*).

VARIETAL SUSCEPTIBILITY OF ONION SEEDLINGS

The writer has reported (*24*) experiments in which *Colletotrichum circinans* caused a damping off of White Globe seedlings under greenhouse conditions. It was of interest to note whether young seedlings of colored varieties would show any signs of resistance, especially since no pigment would have developed at this early stage. Sterilized greenhouse soil was inoculated with a pure culture of the organism, and seeds of a few of the varieties listed above were planted. The percentage of diseased plants in the white varieties were: White Portugal, 85 per cent; Giant White Italian Tripoli, 98 per cent; and Queen, 93 per cent; in the yellow varieties: Michigan Yellow Globe, 100 per cent; and Southport Yellow Globe, 93 per cent; and in the red varieties: Extra Early Red, 81 per cent; and Southport Red Globe, 95 per cent. The essentially equal susceptibility at this stage of all varieties tried, colored and white alike, is self-evident.[6]

The experimental and observational evidence conclusively shows that we have in colored onions a high degree of resistance to smudge but that this character is not present or at least not effective in the young seedlings. As will be shown later, the active resistant principle is a water soluble substance in the dry outer colored scales. This resistance is not to be construed as a case of absolute immunity nor is it unaffected by environmental conditions. This idea is substantiated by the fact that

[6] Under field conditions in our Northern States, the young onion seedlings, being spring-sown, are ordinarily growing in a soil too cool for the development of smudge. This probably accounts for the total lack of any report in America of damping off in the field due to the smudge fungus. In England (1922), however, this organism was found causing a damping off of young seedlings sown in August, at a time when soil temperatures were more favorable. This observation was on White Lisbon variety; no seedlings of colored varieties were available for comparison.

slight infection may occur on colored bulbs and even in rare occasions to a considerable degree if they are unduly exposed after maturity to the bleaching effects of direct sunlight as well as meteoric and soil water.

RELATION OF ONION OIL TO THE PARASITISM OF COLLETOTRICHUM CIRCINANS

The amount of research upon the chemical composition of the onion is limited. According to Remington and Wood (*15, p. 1525–1526*):

> Fourcroy and Vanquihn obtained from the ordinary onion a white acrid volatile substance containing sulphur, albumen, much uncrystallizable sugar and mucilage, phosphoric acid both free and combined with lime, acetic acid, calcium citrate, and lignin. The expressed juice is susceptible of vinous fermentation. The oil is essentially the same in chemical composition as the oil of *Allium Sativum* and consists largely of allyl sulphide $(C_3H_5)_2S$.

According to Gildemeister and Hoffman (*7, p. 546*), Semmler has found that onion oil, contrary to the reports given in most textbooks, consists not of allyl sulphid but of a series of sulphids, chiefly $C_6H_{12}S_2$. Kooper (*11*) found thiocyanic acid in the onion as well and secured positive tests for thiocyanic acid allyl ether, but no trace of acetic, formic, or allyl aldehyde. The germicidal effect of onion juice was noted by Pasteur (*12*).

RELATION OF EXPRESSED ONION JUICE TO COLLETOTRICHUM CIRCINANS

The relation of the constituents of the onion tissue to the fungus was first investigated experimentally by studying the effect of the expressed sap from onion scale upon spore germination. White onion scale tissue was macerated and the sap expressed by forcing through cheesecloth. Portions of the sap were diluted 10, 100, and 1,000 times with distilled water. Spores from a pure culture of the fungus were placed in drops of the expressed sap and in its various dilutions on glass slides in moist Petri dishes. No germination occurred in the sap as expressed from the tissue, nor in the 1 to 10 dilution. In the 1 to 100 dilution fair germination took place, and in the 1 to 1,000 dilution normal germination occurred. This experiment was repeated many times with practically the same results. When bits of fleshy onion scale tissue were added to drops of water containing spores, germination was also inhibited.

In order to determine the effect of heating on this inhibitive substance, a small amount of onion extract was secured in the usual way and divided into two equal parts, one of which was placed in live steam for 20 minutes. No germination occurred in the fresh extract as usual, while in the heated extract a high percentage of the spores germinated normally. From the results of these experiments it seemed quite certain that there was present in the extract a toxic substance which was readily removed by heat.

Since germination of the spore normally takes place in the soil or in water outside the plant, and not in contact with the host cell sap, and since the fungus would thus not come in contact with the inhibitive substance until its mycelium had penetrated the host, the question arose whether this toxic substance within the cell is capable of inhibiting the growth of the germ tube. Spores were germinated in distilled water until the germ tubes were several times the length of the spore, then a number of them were transferred to onion sap extract. The growth of the latter group was checked at once, while those left in water continued to grow at the normal rate. This showed that the mycelium could be checked by the

inhibitive substance of the cell sap. It seemed highly probable that this substance present in the living cells of the host was responsible, in part at least, for the checking of the advance of the fungus after it had penetrated the fleshy scales. It seems logical, therefore, to conclude that the restricted parasitism of the fungus is not due entirely to substances lacking in the fungus, but perhaps in some measure to the toxicity of the cell sap of the host.

EFFECT OF THE VOLATILE ONION OIL ON THE FUNGUS

Experiments were next undertaken to determine the effect of the volatile oil of the onion upon the germination and growth of the fungus. For the germination studies clean glass slides were laid in Petri dishes, two slides in each dish. The expressed juice of white onions, diluted to various degrees as in the above experiments, was used. Two drops of this medium containing the spores were placed on one slide in each Petri dish, while on the second slide in each dish were placed two drops of distilled water containing spores in suspension. The data secured from this experiment are tabulated in Table II.

TABLE II.—*Effect of volatile oil of onion extract on germination of spores of Colletotrichum circinans*

Petri dish No.	Slide No.	Medium.	Dilution.	Germination.	
				Experiment 1.	Experiment 2.
				Per cent.	*Per cent.*
1	1	Onion extract	Undiluted	0	0
	2	Distilled water		0	0
2	3	Onion extract	1 to 10	0	0
	4	Distilled water		75±	25±
3	5	Onion extract	1 to 100	75±	25±
	6	Distilled water		75±	95±
4	7	Onion extract	1 to 1,000	75±	95±
	8	Distilled water		75±	95±

The unusual and striking feature of these experiments is that in the Petri dishes containing the undiluted extract, no germination whatever occurred, either in the extract or in the drops of distilled water. This could be attributed only to the fact that a volatile substance from the expressed onion juice had been absorbed by the distilled water and had prevented the spores from germinating. The toxicity of the onion oil was thus clearly demonstrated. The toxic effect of the onion oil upon the spores in distilled water decreased as the onion extract was diluted. These experiments were repeated many times with essentially the same results.

The effect of the volatile oil was demonstrated in another way. A suspension of spores was made in two tubes of melted potato agar, which were then poured into sterile Petri dishes. After the agar had hardened the plates were inverted. In the center of the inside of the cover of one plate a small amount of onion extract was placed. The agar was thus exposed to the volatile oil, but was not in contact with the extract. The other dish served as a control (Pl. 2, B). In it the spores

germinated normally and growth soon covered the plate. In the other plate the volatile oil prevented germination of all the spores except those around the edge of the plate. In a few days the extract was decomposed by bacteria, since no attempt was made to keep it sterile; then the growth of the fungus proceeded from the margin toward the center of the plate. The fact that the spores in the center of the plate did not germinate showed that they had been killed by the volatile oil.

Another experiment was performed to determine the effect of the volatile oil on the growth of the mycelium. Three plates of potato agar were poured, and to the center of each was transferred a bit of mycelium from a pure culture of the fungus. The colonies were allowed to develop for four days, when they measured 18, 19, and 15.5 mm. in diameter, respectively. One plate was then designated as a control. Within the inverted covers of the other two were placed small amounts of onion extract as described in the previous experiment. In Table III are given the measurements of the colonies for the next five days. The colony in the control plate continued to grow at a normal rate, but those colonies exposed to the volatile oil practically ceased growth. At the end of the third day the extract was removed from one plate; the fungus colony in that plate resumed growth, showing that the mycelium had not been killed, but had merely been checked by the volatile oil. In the third plate a slight amount of growth had taken place by the fifth day, after which, as the onion extract decomposed, the fungus resumed the normal growth rate. This experiment shows that the growth of mycelium is markedly checked in the presence of the volatile oil, but that, unlike the spores, its growth processes are resumed when the inhibitive substance is removed. This fact is of significance in considering the effect of the onion oil upon the fungus hypha invading the scale tissue. One might expect in this case a slowing up or cessation of growth of the hypha, temporarily at least, but not necessarily a killing of the fungus filament.

TABLE III.—*Effect of volatile oil from onion extract on growth of Colletotrichum circinans*

Time of measurement.	Diameter of thalli.		
	Control.	Exposed.	Exposed.
	Mm.	*Mm.*	*Mm.*
Beginning of experiment	18	19	15.5
Second day	27	19	15.5
Third day	33	19	15.5
Fourth day	39	[a] 20	15.5
Fifth day	41	23	16.0

[a] Extract removed at end of third day.

ONION OIL FROM RESISTANT AND FROM SUSCEPTIBLE VARIETIES

It should be kept in mind that the foregoing experiments were conducted entirely with extracts from white onions, which have been shown to be susceptible to the disease. It was of interest to know what degree of toxicity was to be found in the extract from the yellow and the red varieties, the so-called resistant types. Accordingly, extracts were made from Red Globe and Yellow Globe bulbs, as well as from the White Globe, a single bulb being used in each case. It should be pointed out

here that there is a certain amount of pigment present in the epidermal cells of the colored fleshy scales which was necessarily included in the extract. Moreover, it will be shown later that the water extract of dry pigmented scales is toxic to the fungus. It is believed, however, that in these fleshy scale extracts the pigments were so dilute as to have had little or no effect. Various dilutions of the fleshy scale extracts in distilled water were secured, as before, and their effect upon germination of spores was determined. As described in the previous experiment, a Petri dish containing one slide for drops of spores suspended in distilled water and one slide for spores in the onion extract, was used for each dilution. Results of this experiment are included in Table IV.

TABLE IV.—*Toxicity of succulent scale extract from White, Yellow, and Red Globe varieties of onion to the spores of Colletotrichum circinans*

Dilution.	Petri dish No.	Medium.	Spore germination.[1]					
			Experiment 1.			Experiment 2.		
			White.	Yellow.	Red.	White.	Yellow.	Red.
Undiluted	1	Extract	o	o	o	.....	.....	.....
		Distilled water	o	o	o	.....	.....	.....
	2	Extract	o	o	o	.....	.....	.....
		Distilled water	o	o	o	.....	.....	.....
1 to 10	3	Extract	o	o	o	o	o	o
		Distilled water	++	++	++	⊖	⊖	o
	4	Extract	o	o	o	o	o	o
		Distilled water	++	⊕	++	⊖	⊖	o
1 to 20	5	Extract	.....	.....	.....	o	o	o
		Distilled water	.....	.....	.....	⊕	++	⊖
	6	Extract	.....	.....	.....	o	o	o
		Distilled water	.....	.....	.....	⊕	++	++
1 to 40	7	Extract	.....	.....	.....	o	o	o
		Distilled water	.....	.....	.....	++	++	++
	8	Extract	.....	.....	.....	o	o	o
		Distilled water	.....	.....	.....	++	++	+
1 to 60	9	Extract	.....	.....	.....	o	o	o
		Distilled water	.....	.....	.....	++	++	++
	10	Extract	.....	.....	.....	o	⊖	o
		Distilled water	.....	.....	.....	++	++	++
1 to 80	11	Extract	.....	.....	.....	o	+	⊖
		Distilled water	.....	.....	.....	++	++	++
	12	Extract	.....	.....	.....	−	+	⊖
		Distilled water	.....	.....	.....	++	++	++
1 to 100	13	Extract	++	++	++	−	++	⊕
		Distilled water	++	++	++	++	++	++
	14	Extract	++	+	++	⊕	++	⊖
		Distilled water	++	++	++	++	++	++
1 to 1,000	15	Extract	++	++	++	.....	.....	.....
		Distilled water	++	++	++	.....	.....	.....
	16	Extract	++	++	++	.....	.....	.....
		Distilled water	++	+	++	.....	.....	.....

[1] Symbols used: o=no germination; ⊖=0 to 25 per cent spores germinating; −=25 to 50 per cent; ⊕=50 to 75 per cent; +=75 to 90 per cent; ++=90 to 100 per cent.

It is quite evident that but little difference exists between the colored and white Globe varieties used as to the toxicity of their cell sap. In fact, in this respect the differences between individual bulbs of the same

variety are as great as those between varieties. It is to be expected, therefore, that the volatile oil content of these varieties of onions is a factor not necessarily responsible for differences in susceptibility or resistance to smudge.

From the foregoing experiments it may be concluded that the expressed sap from white onion scale inhibits germination and growth of *Colletotrichum circinans* and that this toxicity appears to be due, in large measure at least, to the volatile onion oil which is liberated upon crushing of the tissue. This toxic substance is thermolabile inasmuch as it is largely removed by heating the extract for 20 minutes in live steam. The fact that no appreciable difference in toxicity between sap from colored and that from white Globe scales was found indicates that the volatile oil has little to do with the difference in varietal susceptibility. It is believed, however, that this substance is not to be overlooked as a factor in limiting the parasitism of *C. circinans*.

It is of interest in this connection to note that Bernard (*2*) found the bulb tissue of Loroglossum to have a fungicidal effect upon the mycorrhizal fungi isolated from a number of closely related species of orchid. This was demonstrated by placing a piece of the bulb on agar in proximity to the thallus of the fungus. Growth of the latter did not extend within a certain zone surrounding the bit of orchid tissue.

RELATION OF SCALE PIGMENTS TO DISEASE RESISTANCE

It will not be necessary to review the literature upon plant pigments since it has already been brought together by others, including Wheldale (*25*) and Wakeman (*22*). Although a few cases of correlation between resistance and the red or yellow pigments in plants have been noted (*5, 6, 17, 21*), in no previous case, so far as the writer is aware, has the relation of plant pigments to plant parasites been carefully studied. The fact that a water extract from pigmented onion scales has been proved to be highly toxic (*23*) to the smudge organism, has prompted a further inquiry into this subject. It is needless to point out that the widespread occurrence of closely related pigment compounds in the plant kingdom justifies further investigation of their relation to parasitism.

NATURE OF THE RED AND YELLOW PIGMENTS IN ONION SCALES

The red and yellow pigments of onion scales are solutes in the cell sap of the outer epidermal layer. They first appear when the plants are about half grown and continue to develop during the remainder of the growing season. At maturity the color is most intense in the outer scales which have dried down to a thin papery consistency, and it becomes progressively less marked in the fleshy scales as the center of the bulb is approached. Intensity and color of pigment vary with varieties. Colored varieties may be roughly grouped into the large classes, the yellow and the red. There are numerous varieties, however, such as the Australian Brown, which lie on the border line between the two groups. Pigmented cells of the yellow varieties when treated with alkalies turn deep brownish yellow in color, a reaction typical of the flavones (*25*). The pigmented cells of the red varieties turn pink in acid and green in alkaline solutions, which reactions are characteristic of the anthocyans. Perkin and Hummel (*14*) isolated quercetin, a flavonol,

from a hot water extract of colored onion scales, but they did not state the variety or color of onion used. Perkin and Everest (*13, p. 201*) state that attempts to isolate a quercetin glucoside from onion skins have hitherto failed. Our work shows that quercetin exists to a considerable extent in both the yellow and the red scales, and in the last case it evidently occurs along with an anthocyan. Beyond the work of Perkin, just cited, there is no information available as to the exact nature in which the red and yellow pigments exist in the onion tissue. In connection with the present problem this question is now being investigated in cooperation with the Department of Pharmacy, University of Wisconsin, and results with their bearing upon the relation between parasite and host will be published later. It is a matter of common observation that upon death of the cell, the cell sap pigments readily diffuse out. Likewise in the onion, there is considerable diffusion of soluble pigment from dead outer scales but little or none from the succulent scales.

RELATION OF ONION PIGMENTS TO THE FUNGUS

The close correlation between presence of pigment in the outer scale of the onion and resistance to smudge has already been pointed out. In further search for the substances in the plant responsible for resistance, a study was made of the effect of water extracts of the dry outer scales upon the germination and growth of the fungus.

It has been shown that when bits of the fleshy scale of the white or colored onion were placed in drops of water containing spores of *Colletotrichum circinans*, germination was entirely inhibited. Similar trials made with outer dead scales of white onion yielded no inhibition, however. Thus, it was evident, that a large part of the volatile oil present in the fleshy tissue is lost as the outer scale dries down. It is assumed, therefore, where dry outer scales were used that toxicity of the volatile oil did not enter in to any appreciable degree in the following experiments.

METHODS

The methods used were as follows: Drops of distilled water containing spores of the fungus were placed on clean glass slides, two drops on each slide. Petri dishes lined with moistened filter paper were used as moist chambers; two slides were placed in each Petri dish, one serving as a control, the other as a means of testing the effect of scale extract on spore germination. In the drops on the latter slide bits of onion scale about 2 mm. square were placed, usually two such pieces per drop. Observations were made and recorded after 18 to 24 hours.

EFFECT UPON SPORE GERMINATION

Further experiments, conducted with bits of tissue from the dead outer scales, resulted in quite a different reaction on the part of the fungus than had hitherto been noted. No indication of the injury to control drops so evident in the last experiment was noted, showing that the fungicidal effect of the volatile oil is largely, if not entirely, absent in the dry outer scales. In the case of drops of spore suspension containing bits of the dry white scale, germination occurred and appressoria formed as usual. The behavior of the organism was thus very

similar to that commonly observed in distilled water or on nutrient media. However, where red or yellow scale tissue was added to the drops distinctly abnormal germination occurred. In a large majority of cases, the spore sent out a germ tube, which usually acquired a length of only about one micron, when the wall at its tip either dissolved or ruptured and a portion of the cell contents exuded and collected in a naked mass at the end of the very short and usually indistinguishable tube. Thus at the end of 12 to 18 hours most of the spores were to be found each with a naked mass of the cell contents adjacent to them. This feature is illustrated in figure 1 and Plate 3. For the purpose of the present paper this condition is referred to as "rupturing" of the germ tube, although the exact details of the process have not been worked out and it is realized that further study may necessitate another descriptive term. This phenomenon usually prevented further functioning of the spore.

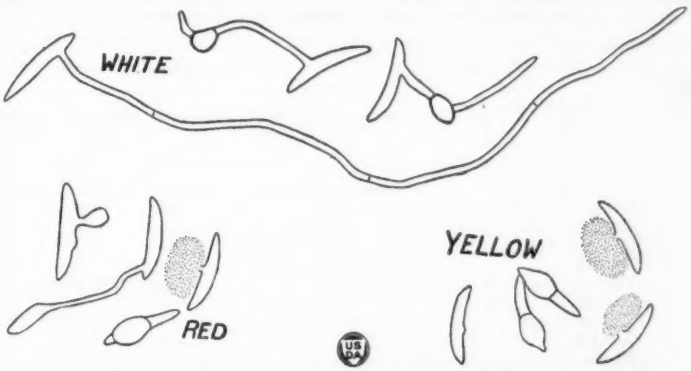

FIG. 1.—Effect of water extract of outer onion scales upon spore germination of the smudge fungus. Bits of dry scales of white, yellow, and red onions were added to drops of spore suspension in distilled waters. Note typical germ tubes and appressoria in the case of the white scale as compared with abnormal germination in the case of the colored scales. The abnormalities are of three types: (1) Ruptured germ tubes, (2) short, thick germ tubes, (3) swelling of spores without production of germ tubes.

In a smaller percentage of cases there occurred other abnormal types of reaction, which may be divided into two general classes. The first class includes those which will be referred to as "swollen spores." In this group there is a marked swelling of the spores, usually at one end, with septation occurring at the limit of this enlargement (fig. 1); no germ tubes are formed. In the second class, designated as having "abnormal germ tubes," the tube grows for a short distance, is usually swollen at the tip or abnormally large in diameter, and never produces an appressorium (fig. 1).

The percentages of various types of germination obtained in the first experiment are recorded in Table V. The results were determined by counting several microscopic fields from each slide. It is to be noted that ruptured germ tubes occurred with a large majority of the spores where red or yellow scale tissue was added to the drops. Moreover, the spores reacted essentially alike to the red and yellow tissue. Numerous repetitions of this experiment yielded essentially similar results. The data from one of these experiments are recorded in Table VI.

TABLE V.—*Effect of dry outer scale tissue of red, yellow, and white varieties of onion upon the spore germination of Colletotrichum circinans (experiment 1)*

Variety.	Germination.				
	No germination.	Normal germination.	Ruptured germ tubes.	Abnormal germ tubes.	Swollen spores.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Red	0	21	78	0	1
Yellow	0	35	61	2	2
White	10	90	0	0	0

TABLE VI.—*Effect of dry outer scale tissue of red, yellow, and white varieties of onion upon the germination of Colletotrichum circinans (experiment 2)*

Petri dish No.	Color of tissue used.	Germination.				
		No germination.	Normal germination.	Ruptured germ tubes.	Abnormal germ tubes.	Swollen spores.
		Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
1	Red	7	2	45	42	4
	Control	9	91	0	0	0
2	Red	12	0	88	0	0
	Control	12	88	0	0	0
3	Yellow	34	0	66	0	0
	Control	7	93	0	0	0
4	Yellow	24	0	76	0	0
	Control	11	89	0	0	0
5	White	8	92	0	0	0
	Control	9	91	0	0	0
6	White	13	87	0	0	0
	Control	47	53	0	0	0

EFFECT UPON GROWTH

The experiments just described show conclusively that the water extract of pigmented dry scales is highly toxic to spores of *Colletotrichum circinans*, while a similar extract from white scales has no such inhibitory effects. The fact that the toxic substance (or substances) is so readily diffusible leads one to surmise that the plant may be protected by its gradual solution into the soil water immediately surrounding the bulb. The smudge organism develops normally in the soil; hence it is conceivable that in some cases at least the spore may germinate at some distance from the bulb; thus the mycelial hyphae may be the first to come into close contact with the bulb or with the soil solution immediately surrounding it. Accordingly an experiment was carried out to determine the effect of the pigmented extract upon the growth of the fungus mycelium.

Spores were germinated in water until the germ tubes were several times the length of the spores. Small pieces of dry outer red scales were then added to certain of the drops, while others were left as controls. After 24 hours, examination showed that whereas the hyphae in the control drops had continued to grow normally, those to which the bits of red scale had been added had grown little if at all from the time these pieces

were added. This is best shown in figure 2, which contains camera-lucida sketches of representative spores and thalli, made at the time when the scale tissue was added to the drops of spore suspension and at the conclusion of the experiment. It is quite evident that the toxic substance (or substances) here concerned inhibits the growth of hyphae as well as the normal germination of spores of *Colletotrichum circinans*.

EFFECT OF COLORED AND OF UNCOLORED PORTIONS OF THE SAME SCALE

It has already been pointed out that ordinarily little or no pigment is formed in the outer scales at the neck of the bulb. Moreover, under suitable conditions this particular portion of an otherwise resistant plant is readily attacked. It was important, therefore, to know whether the inhibitive substance already demonstrated in the pigmented tissue could be found in this closely adjacent uncolored area. The outer scale of a red onion set (Red Wethersfield variety) which had become naturally infected in the uncolored portion at the neck was carefully removed.

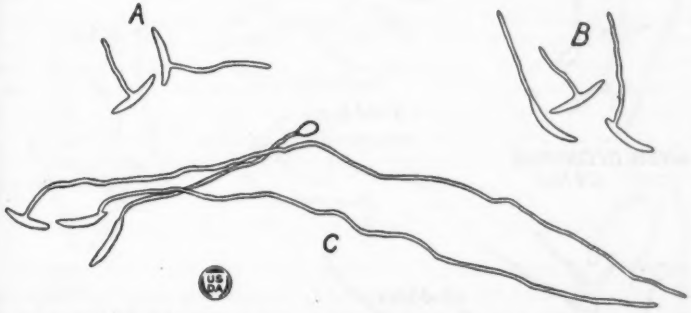

FIG. 2.—Effect of colored outer scale extract upon growth of hyphae of the smudge fungus. Spores were germinated in drops of distilled water to the point illustrated by camera lucida sketches in A. To some of the drops bits of red outer scale were then added. Growth was recorded by further sketches 24 hours later. In B are shown typical spores from drops to which red scale tissue has been added, while in C are shown typical spores from control drops. Growth was almost, if not entirely, checked when the scale tissue was added.

Bits of tissue were then cut from the colored part of the scale just below the area of infection, and from the uncolored part about 1 inch above this point. The scale after the removal of these bits of tissue is shown in Plate 4. The bits of tissue from the two points were placed in drops of spore suspension according to the previously described method. In the drops containing uncolored tissue normal germination occurred, while in those containing colored tissue practically all of the spores developed ruptured germ tubes. Representative spores from each lot are illustrated in Plate 4. The experiment has been repeated several times with similar results, showing conclusively the close correlation existing between the pigment and the toxic entity.

EFFECT OF DILUTING THE PIGMENT EXTRACT

In order to secure a more nearly uniform basis of comparison between white, yellow, and red bulbs, an equivalent amount of dry scale tissue from each variety was weighed. The three lots were then allowed to steep for 24 hours at room temperature in distilled water added at the

rate of 20 cc. per gram of tissue. The extract was filtered and various dilutions of the filtrate with distilled water were used as media for spore

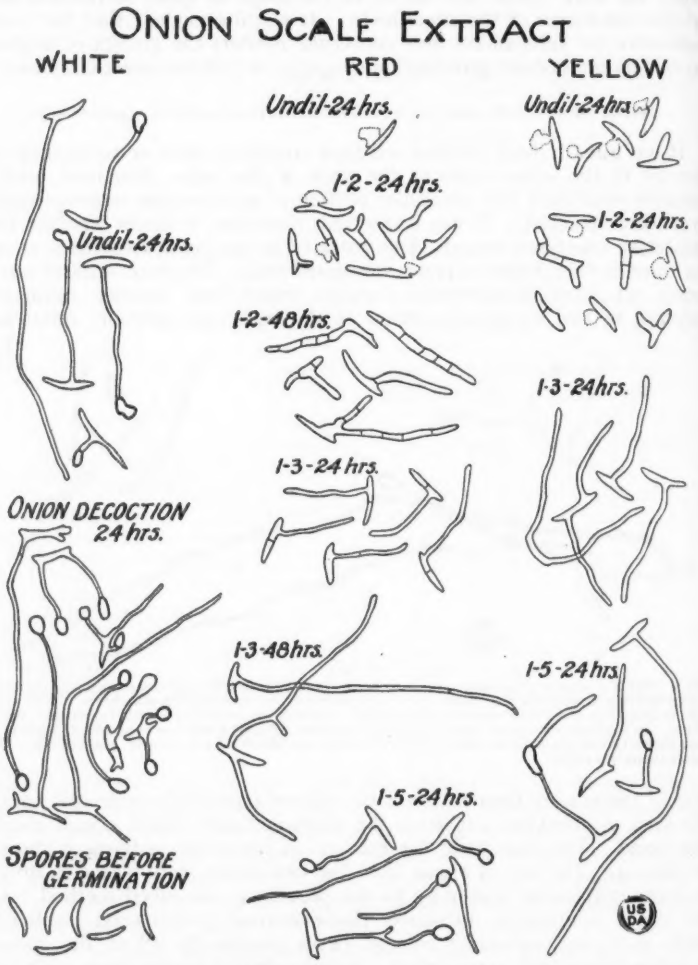

FIG. 3.—Spore germination of *Colletotrichum circinans* in extracts made from dry outer scales of white, red, and yellow varieties of onion, and in onion decoction. One gm. of dry scale was extracted over night in 20 cc. of distilled water at room temperature. Dilutions were then made up to 1 to 5 with distilled water. Note that germination and growth were normal in onion decoction and undiluted white scale extract, while in the undiluted colored scale extract typical abnormal germination occurred as shown in fig. 1. With dilution the toxicity of the extract was reduced, however, quite normal germination occurring at 1 to 5. Normal ungerminated spores are shown in the lower left-hand corner. See further explanation in the text.

germination. The results are recorded below; representative spores from each dilution are illustrated by camera lucida sketches in figure 3.

White scale tissue:

Undiluted—Good germination, growth, and appressorium formation.

Yellow scale tissue:
- Undiluted.....At 24 hours, a large percentage of ruptured germ tubes; at 48 hours, no change.
- 1 to 2.....At 24 hours, many ruptured germ tubes and many short, thick, germ tubes; at 48 hours, practically no change.
- 1 to 3.....At 24 hours, short, thick germ tubes, no appressoria; at 48 hours, good growth and appressorium formation.
- 1 to 5.....At 24 hours, good growth and appressorium formation.

Red scale tissue:
- Undiluted.....At 24 hours, very little germination; when it occurred it resulted in ruptured germ tubes; at 48 hours, no change.
- 1 to 2.....At 24 hours, ruptured and short, thick, abnormal germ tubes; at 48 hours, some additional growth of latter.
- 1 to 3.....At 24 hours, germ tubes slightly longer than in 1 to 2, no appressoria, an occasional ruptured germ tube; at 48 hours, considerable additional growth, but no appressoria.
- 1 to 5.....At 24 hours, good germination and appressorium formation.

It will be seen that in the undiluted extract of white scales, germination was quite normal and compared favorably with that in onion decoction. In the undiluted red and yellow scale extract "ruptured" germ tubes were common. As the colored extracts were diluted, however, there was a gradual diminution of the toxic effect with seemingly normal germination occurring in the 1 to 5 dilution This shows that the inhibition in the concentrated solutions was not due to a lack of nutrients. Moreover, the toxic entity was apparently about equally concentrated in the extract from the red and that from the yellow scales, if we may judge from the extent to which germination was reduced in equal dilutions of the two extracts.

RELATION OF CELL SAP ACIDITY TO THE FUNGUS

The reaction of the cell sap of the host plant as a factor in resistance to parasites has been emphasized by Comes (*3*, *4*), but Jones, Giddings, and Lutman (*10*), Hawkins and Harvey (*9*), Vavilov (*20*), and others found no positive evidence of its importance as the cause of resistance. Since this point is not one to be overlooked, a study of the acid toleration of *Colletotrichum circinans* was made.

Onion decoction, a medium which had proved very favorable for germination and growth, was used in this study. Two hundred gm. of fresh onion bulb tissue and 1,000 cc. of distilled water were cooked in the steamer for one hour. This decoction was then filtered through filter paper and the hydrogen-ion concentration of the filtrate determined. The medium was then divided among ten 200-cc. flasks and the acidity of each adjusted with standard solutions of hydrochloric acid or with sodium hydroxid so as to cover the following range of P_H values: 1.8, 2.0, 3.0, 3.6, 4.4, 5.2, 6.4, 7.4, 8.8, and 9.4. A portion from each flask was used for spore germination tests and the remainder sterilized for 30 minutes at 7 pounds pressure. The acidity of each flask was again tested after sterilization and the following values noted: 1.6, 1.8, 2.2, 3.4, 4.6, 5.2, 6.2, 6.8, 7.4, and 8.2. Spore germination tests were also made with portions from these lots after sterilization. Good germination occurred in the alkaline media and in the acid up to P_H 3.4. When the acidity increased beyond that point, germination was reduced and abnormal. In the unsterilized extract at P_H 1.8 and 2.2, 10 to 15 per cent of the spores began to germinate, but the germ tubes appeared to rupture and exude cytoplasm in a manner strikingly similar to that observed in the water extract from dry

colored onion scales. Occasionally there was a slight swelling of the spores (fig. 4). At P_H 3, about 43 per cent of the spores germinated, but the hyphae were abnormal in size and stunted, and in many cases the germ tubes ruptured. In the sterilized extract no germination occurred at P_H 1.6 to 2.2, except for an occasional ruptured germ tube at P_H 2.2.

The growth of the fungus on onion decoction of various P_H values was tested. The medium was made as described above, except that 2 per cent dextrose was added. Duplicate portions of 75 cc. each in 300-cc. Jena glass flasks were used. The medium was inoculated by transferring a piece of stroma and mycelium from a potato agar culture of the organism to each flask. The flasks were kept in diffused light at room temperature (20° to 25° C.) for 54 days. The mycelial growth was then removed from the remaining culture medium by filtration and the reaction of the filtrate determined. The P_H value of the medium before and after filtra-

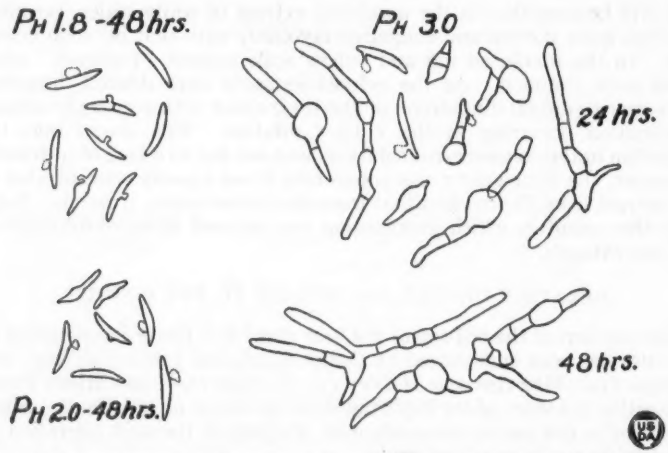

FIG. 4.—Spore germination of *Colletotrichum circinans* in onion scale decoction adjusted to the various degrees of acidity indicated. Note the ruptured germ tubes at P_H 1.8 and 2.0, and the same together with swollen spores and short thick germ tubes at P_H 3. See further explanation in the text.

tion, and at the end of the experiment, as well as the amount of fungus growth occurring in the flasks, are recorded in Table VII.

A repetition of this experiment with Pyrex flasks and the use of spores instead of mycelium as inoculum yielded essentially the same results.

It may be concluded from the above experiments that both the spores and the mycelium of *Colletotrichum circinans* germinate and grow well in onion decoction ranging in P_H value from about 3.5 to 8. As the actual acidity increases beyond 3.5 there is a sharp reduction in germination and growth, with a tendency toward rupturing of the germ tubes. It is important also to note that in the flask cultures, where good growth occurred, the reaction of the medium changed decidedly toward the alkaline during the period of the experiment. The rapidity of this change and the actual variations in the reaction from day to day were not determined.

The acid toleration of the fungus having been studied, the reaction of the onion tissue was next considered. Plants of the Red, Yellow, and White Globe varieties growing out-of-doors were pulled at about 8.30

a. m., July 31, 1920, and determinations made directly by the electrolytic method.[6] The tops and the scales were chopped up separately and the expressed juice used for the determinations. The results are given in Table VIII. It is to be remembered, of course, that this method does not necessarily give the actual reaction which the fungus hyphae encounter as they invade the plant. It is conceivable that there may be natural variations in reaction in different parts of the bulb and at different hours of the day, while secretions of the fungus itself might change its immediate surroundings. Valleau (*19*) suggests in the case of brown-rot of plums (*Sclerotinia cinerea*) that, "it is very probable that in the slow development of the fungus in the resistant fruits enough oxalic acid is produced by the hyphae to actually become toxic to them." However, there was no evidence of wide variations in acidity between resistant and susceptible onion bulbs, and the acidity of the juice expressed from either was much lower than that of the onion decoction in which germination and growth of the fungus were inhibited. Moreover, the fungus tended to change very acid solutions to a more alkaline, and thus more favorable, reaction.

TABLE VII.—*Growth of Colletotrichum circinans in onion decoction adjusted to various P_H values (duration of experiment, 54 days)*

Flask No.	P_H value.			Amount of growth.
	Before sterilization.	After sterilization.	At end of experiment.	
1	2.0	1.6	1.8	None.
2	2.0	1.6	1.8	Do.
3	3.0	3.0	2.0	Do.
4	3.0	3.0	2.0	Do.
5	3.2	3.0	2.6	Very slight.
6	3.2	3.0	2.6	Do.
7	3.8	3.6	5.2	Good growth and sporulation.
8	3.8	3.6	4.8	Do.
9	4.6	4.6	6.2	Do.
10	4.6	4.6	6.2	Do.
11	5.2	5.4	7.2	Good growth.
12	5.2	5.4	7.0	Do.
13	6.4	6.4	7.4	Do.
14	6.4	6.4	7.4	Do.

TABLE VIII.—*Acidity of expressed juice of scales and tops of Red, Yellow, and White Globe onions*

Variety.	P_H values.	
	Tops.	Succulent scales.
Red Globe	5.2	5.3
Yellow Globe	4.8	5.4
White Globe	5.2	5.4

[6] The writer is indebted to Mr. O. C. Bryan of the Department of Soils, University of Wisconsin, for making the hydrogen-ion determinations.

The possibility of a connection between acidity and toxicity in cold water extracts of dry outer scales was considered. Outer scales of red, yellow, and white bulbs were extracted for 24 hours in redistilled water having an approximately neutral reaction. For each gram of tissue 15 cc. of water were used. The hydrogen-ion concentration of each extract was then determined and portions of each were used for spore germination tests. The results are given in Table IX. Although the acidity of the outer scale extracts is somewhat higher than that of the succulent tissue, it was still in all cases within the range tolerated by the fungus, and the toxicity, therefore, must be due to something beside acidity. In none of these determinations was there any evidence of wide variation in acidity between resistant and susceptible bulbs.

TABLE IX.—*Acidity of cold water extracts of dry outer scales of white and colored onions*

Variety.	P_H value of extract.	Reaction of spores.				
		Ungerminated.	Normal germination.	Ruptured germ tubes.	Swollen spores.	Short, abnormal germ tubes.
		Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Yellow Globe	3.70	52	0	47	1.0	0.0
Yellow Globe	4.67	24	0	74	1.0	1.0
Red Globe	3.55	91	0	8	0.5	0.5
White Globe	4.03	Percentage germination fairly high; good growth; numerous appressoria; new conidia being formed at 48 hours, making spore counts impossible.				

DISCUSSION OF RESULTS

The resistance of the common colored varieties of onions to smudge is well established. The water extracts from the dry outer scales of red and of yellow onions have shown a marked toxicity to spores and mycelium of *Colletotrichum circinans*, and the reaction of the fungus is quite different from that brought about by the onion oil. In the scales of white varieties the toxic entity ordinarily associated with flavone or anthocyan pigments in the colored varieties, is apparently absent or too slight to be detected. Moreover, in the colored varieties, it is confined to that tissue in which there has been production of pigment. Its absence in the unpigmented portions of the colored scales about the neck of the bulb is correlated with a marked susceptibility to the disease in this limited region. The fact that this toxic substance is readily diffusible in water indicates that meteoric or soil water immediately adjacent to colored bulbs may become sufficiently toxic to ward off invasion by the fungus. In any case, we are led strongly to suspect that by means of this toxic substance the dry outer scales of colored bulbs serve as a barrier between the parasite in the soil and the underlying fleshy scales, which have been shown to be susceptible to infection.

The exact identity of the toxic substance (or substances) remains to be determined. Its very close association with the scale pigments is suggestive. The toxicity may be due to the color compounds or to compounds closely associated with them. Attempts are now being made to isolate the substance in pure form, but it must be recognized

that such an analysis breaks down to some extent the complex balance of compounds as they exist in the tissue.

The marked toxicity of the expressed juice of fleshy scales of both the susceptible and the resistant varieties is also of interest. It seems to be due largely to the volatile onion oil, but apparently it does not contribute to the noted differences in varietal susceptibility of the northern Globe types. The juice from colored scales and that from white scales are essentially equal in toxicity. Moreover, as stated above, infection of the fleshy scales seems to progress as readily in resistant as in susceptible varieties. The relation of the volatile oil to the aggressiveness of the parasite may well be considered, however. The smudge organism causes an exceedingly slow decay of the bulb compared with certain other bulb parasites, such as *Botrytis allii*, for instance, although penetration by the former is accomplished very readily and easily and in fact much more generally than in the case of the latter fungus, which usually requires a wound in order that invasion may take place. When we consider the marked toxicity of the onion oil to the smudge fungus, we are led to wonder that invasion occurs at all. However, several possible explanations at once present themselves. In the first place, the oil as it is released from the expressed sap may be in a different state than that in which it actually exists in the living cell. In the allied case of the mustard oil of the crucifers, allyl isothiocyanate, for instance, the oil exists in certain cells as the glucoside, sinigrin, while in adjoining cells is contained a glucoside-splitting enzym, myrosin. When the tissue is crushed the enzym comes into contact with the glucoside and the volatile oil is released. Does a similar condition prevail in the onion? In the second place, under ordinary natural conditions the mycelium of the smudge fungus apparently does not invade the living host cells, but weakens and kills them, usually slightly in advance of the hyphae, by means of some diffusible substance. Moreover, the change in color and texture of the cell contents and, in the case of red scales, the tendency toward formation of coloring matter in the parenchymatous cells both indicate certain effects on host metabolism ahead of the parasite. It may be that through these changes in the cells the volatile oil is either broken down or released and hence that its toxic effects are reduced, at least to the point where invasion is merely retarded and not entirely checked.

The study of penetration indicates that the fungus is capable of penetrating the cuticle and dissolving the cellulose of the walls equally well in all varieties. The acid range tolerated in culture media is wider than the range occurring in the host. It is assumed, therefore, that differences in cell membranes and in acidity of cell sap do not materially affect resistance to the smudge organism.

SUMMARY

(1) Onion smudge is a common disease of the bulb scales. In general, white varieties are susceptible to the disease while colored varieties show a high degree of resistance.

(2) When the dry outer scales of colored varieties were removed the fleshy scales were found to be very susceptible, invasion taking place in the same manner as in the white varieties. The highly colored dry outer scales apparently serve as a barrier to the entrance of the parasite.

(3) In the seedling stage all varieties tried were equally susceptible.

(4) The expressed juice of succulent onion scales is highly toxic to the fungus; this effect is seemingly due in large measure to the volatile onion oil. The extracts from resistant and from susceptible varieties have essentially equal degrees of toxicity. The onion oil apparently is not a factor contributing to varietal resistance to smudge in the northern Globe types, but its importance in limiting the parasitic action of the fungus is suggested.

(5) The red and yellow colors in onion bulbs are due to pigments which are present in the cell sap of the outer epidermal layer. This color is most intense in the dry outer scales, and at least a portion of the pigment in these is readily soluble in water.

(6) Water extract of dry outer pigmented scales causes abnormal germination and retards growth of the fungus, while that from dry outer white scales does not. Essentially the same reaction was recorded with the yellow as with the red scales.

(7) A substance (or substances) present in the dry outer scales of resistant bulbs and closely associated or identical with the red and yellow pigments is apparently the chief factor causing resistance to smudge. Studies are now being directed toward isolation and identification of the toxic entity.

(8) Within the range determined for host tissue, variation in the hydrogen-ion concentration of onion decoction as a medium affects neither germination nor growth of the fungus.

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PLATE 1

Varietal resistance to onion smudge.

White, yellow, and red onions grown in the same field of smudge-infested soil and gathered shortly before harvest. In the case of the white (susceptible) variety the fungus has already developed abundantly on the outer scale of bulb and neck. In the red and the yellow (resistant) varieties, the colored outer scales have remained free from the disease; infection has occurred only at the neck, where little or no pigment developed.

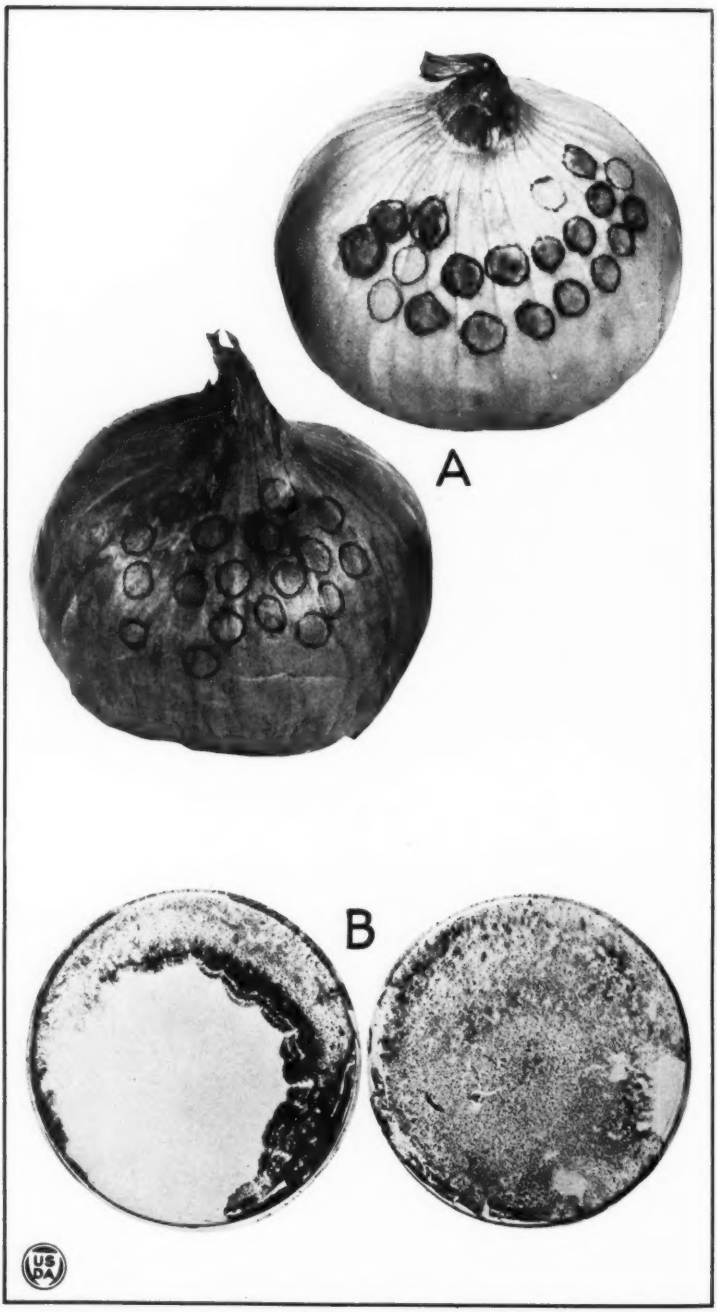

A
B

PLATE 2

Relation of volatile oil and scale pigment to the onion smudge organism.

A.—Relation of outer colored scale to infection: Two bulbs of Yellow Globe (resistant) variety inoculated by placing drops of spore suspension, in onion decoction, at points indicated by black circles.

Left: Inoculations made on dry, highly pigmented outer scale; no infection resulted.

Right: The thin, dry, outer pigmented scales were removed from this bulb and the inoculations were made on the first fleshy scale, which contained relatively little pigment; scale infection resulted in a majority of cases.

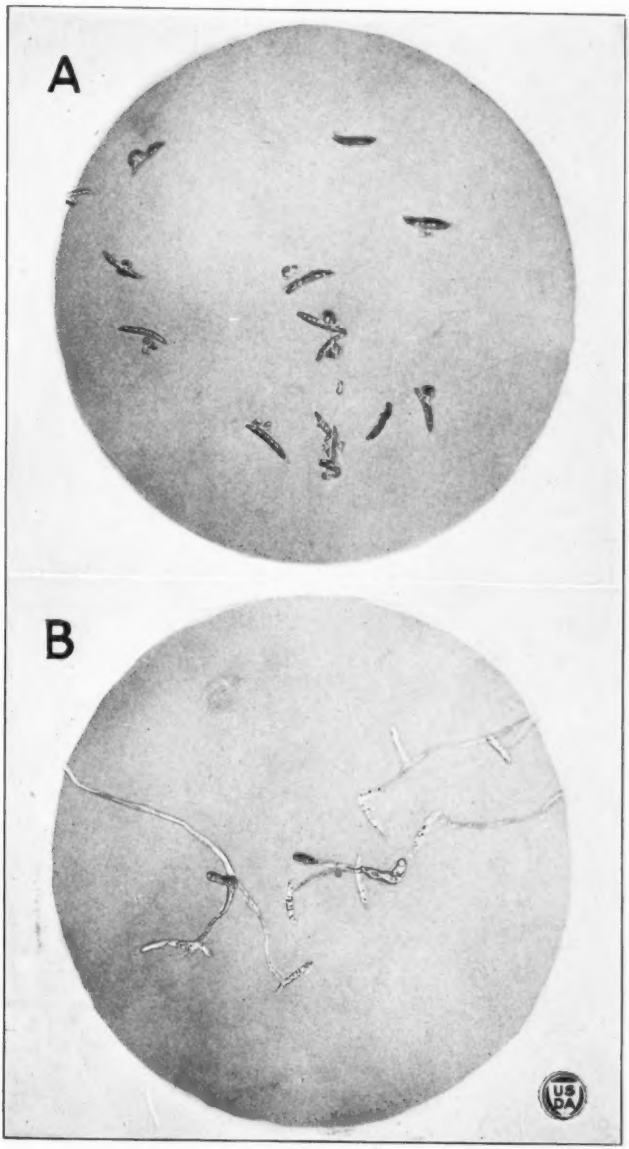
B.—Effect of volatile oil upon spore germination: Petri dish cultures prepared by pouring melted potato agar in which spores of the smudge organism were suspended. Plate at left exposed to volatile oil of onion by being inverted over a small amount of expressed onion juice for several days. The spores were all killed except those around the outer edge of the plate. As the juice deteriorated due to bacterial contamination, growth progressed inward from the unaffected area. Plate at right untreated; normal germination and growth occurred thoroughout.

PLATE 3

Effect of cold water extract from dry outer onion scales upon spore germination of *Colletotrichum circinans*.

A.—Photomicrograph of spores about 17 hours after being placed in an extract from the yellow scales. Note the masses of naked cytoplasm which have exuded from the spores after the tip of the young germ tubes have ruptured. Compare with B.

B.—Photomicrograph of spores about 17 hours after being placed in an extract from white scales. Note the normal germ tubes and appressoria. Compare with A. See also figure 1 and further explanation in the text.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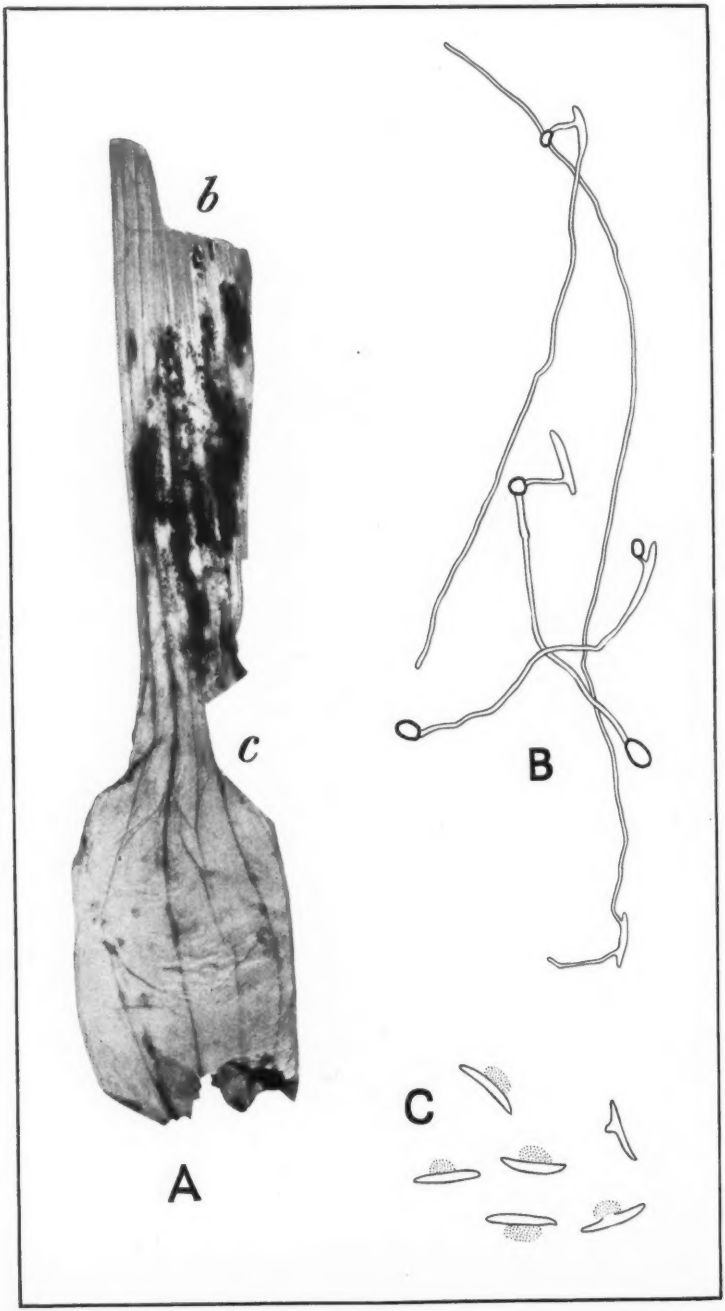
b
c
A
B
C

PLATE 4

Correlation of pigment with onion smudge infection and with the occurrence of the toxic entity.

A.—Portion of outer scale removed at harvest time from a red onion set similar to that shown in Plate 2. (Enlarged × 3.) Note that heavy smudge infection occurred at the neck above the point where pigment developed, while below that point the scale remained free from the disease. The scale tissue in the respective portions was tested for the presence of the toxic entity. Results of spore germination tests are shown in B and C.

B.—Normal germination resulting when bits of uncolored portion of scale from *b* in A were added to a drop of distilled water in which spores were suspended.

C.—Abnormal spore germination similar to that shown in figure 1, resulting when bits of the colored portion (*c*) of the same scale were placed in another drop of spore suspension. Note "rupturing" of germ tubes or production of short tubes of abnormally large diameter.

THE EFFECT OF RESPIRATION UPON THE PROTEIN PERCENTAGE OF WHEAT, OATS, AND BARLEY[1]

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INTRODUCTION

Scientific literature contains considerable information dealing with the protein content of small grains. In a review of the available information bearing upon this subject, which has accumulated during the past century, one chief point of interest seems to be the variation in protein percentage found in the different grains when produced under different conditions of soil and climate.

Much study and investigational work has been conducted to ascertain the cause of the variation found in this regard, especially with wheat. Wheat has been the subject of greatest study, because of the interest of those engaged in the manufacture of wheat products, where the chemistry of the grain is of paramount importance in determining the quality of the output.

The reason why grain produced under a given environment will yield a berry entirely different in chemical structure from that produced under a condition of a different nature, has been a subject of much study with the grain chemist as well as with those engaged in physiological investigations.

It seems to be the generally accepted idea at the present time that the percentage of protein in wheat is due to a certain environmental condition which prevails during the growth and maturity of the grain. Upon analysis of the grain, grown under widely different environmental conditions, this is generally found to be true. It is common knowledge that wheats grown in the north central section of the United States are of a higher protein percentage and milling value than those grown in the eastern or far western sections. There is no doubt that the varieties being grown for the highest total yield in these respective areas, account, to a certain degree, for the difference in protein percentage in the grain. But when consideration is confined within a variety, the variation is found to be very wide.

Some investigators have attributed the difference to the available nitrogen in the soil, others to varietal differences, while most students of the subject have recognized it to be a physicochemical phenomenon correlated more directly with the climatic conditions which prevail during the life processes. In general, the protein percentage has been found to be dependent somewhat upon the length of the growing season and to a greater degree upon the length of the ripening period. As these two factors are dependent largely upon the amount of available moisture in the soil, the strongest correlation has been found between the amount of rainfall, its distribution, and the protein percentage.[2]

[1] Accepted for publication Feb. 20, 1923. Published with the approval of the Director as paper 397 of the Journal Series of the Minnesota Agricultural Experiment Station.

[2] McGINNIS, F. W. THE RELATIVE EFFECT OF ENVIRONMENT UPON THE CHEMICAL COMPOSITION OF WHEAT, OATS, BARLEY, AND CORN. Unpublished thesis. Copies on file in Cornell University Library, Ithaca, N. Y. 1918.

That the external environmental conditions are responsible largely for the protein percentage found in the grain has been noted from the earliest observations. A realization, however, of the manner in which the contents of the grain are influenced by the capricious nature of external conditions and the physiological complexities which may arise during the life of the plant, is of more recent discovery. In order to understand fully how an environmental condition may affect a variation in the chemistry of the grain, it is necessary to follow out, from the earliest stages, the development of a kernel and the processes through which it reaches maturity.

Early investigational work along this line consisted of chemical analyses of the grain being made shortly after the time of flowering or when the mold of the grain was first formed, and continuing progressively at short intervals until the grain was mature. The nitrogen percentage was found to be highest as the kernel mold was formed and became decreasingly less as the grain filled and approached maturity. These observations led investigators to assume that most, or practically all, of the nitrogenous material was taken from the soil and moved into the grain during the earlier stages of growth, and that the filling of the endosperm with starch or carbohydrate material was carried out in the later stages of growth. Thus, it was concluded that the percentage of protein would be influenced directly by the amount of carbohydrate produced and translocated during the filling stage. As the environmental conditions in most of the grain sections are favorable to maximum growth during the early stage of development, and very often too severe to permit later growth to proceed or the grain to be filled, this was the natural conclusion to be drawn.

More recent work by Brenchley (*2*)[3] of the Rothamsted Experiment Station, Thatcher (*5, 6*), of the Minnesota Agricultural Experiment Station, and others, has brought out some slightly different ideas on the subject. These investigators have shown that the greater amount of nitrogenous material of the wheat or barley kernel is not taken up during the early stage of growth, but that the nitrogen inflow is continuous until the grain has reached maturity. According to Brenchley the nitro-carbohydrate movement into the grain is in constant ratio during the entire period of kernel development. It has been suggested that the variation in protein content is not due to the failure of the plant to produce and translocate carbohydrate material at the later stages of growth, but that the percentage of nitrogen found in the kernel is determined by the amount of carbohydrate lost by respiration during the desiccation process throughout the ripening period.

The difference of opinion as to which process is most effective in determining the character of the grain contents suggested the necessity of obtaining definite data to verify the later idea, which appears from available information to be an unwarranted conclusion. The authors have conducted a project through the years 1920 and 1921 to determine the variation in protein in wheat, oats, and barley, as affected by respiration, and to show the relative variation in protein when the same grains are grown under widely different conditions.

[3] Reference is made by number (italic) to "Literature cited," p. 1048.

PLAN OF EXPERIMENT

Marquis wheat, Improved Ligowa oats, and Manchuria barley were grown in 17 localities in the United States during the years 1920 and 1921. The grains were grown at experiment stations and substations in eight States which represent extremes, so far as the quality of grain produced is concerned. The seed was sent to each station from University Farm, St. Paul, Minn., each year, so that the seed planted would be identical. Plantings were made at the regular planting time in the spring and harvested when the grain was mature. Samples of the crop in each instance were sent to University Farm, where nitrogen determinations were made for total protein.

During the summer of 1921, respiration studies were made upon these grains during the ripening or desiccation period at University Farm, to ascertain the amount of material lost during this time. The studies were started when the grains reached the dough stage, before the desiccation process of maturity had begun, and continued until complete maturity.

Each morning at 8 a. m. samples of head material of each of the grains were collected, placed in an air-tight container, and taken to the laboratory. Twenty-five grain samples were picked from the spike by hand and immediately placed in a respirometer. The grains were subjected for 2 hours to a temperature of 30° C., after which the carbon dioxid respired was swept through the modified Truog absorption tower and collected in saturated barium hydroxid solution. The carbon dioxid evolved in this time was calculated in milligrams by titration with *N/10* hydrochloric acid and computed into carbon dioxid lost per 100 gm. of dry matter in 24 hours.

Table I shows the results of this work. It should represent the maximum loss by respiration which could be expected under field conditions. A constant temperature of 30° C. would average the high rate of respiration during the heat of the day and the lower rate during the cool night time.

Table I shows the amount of carbon dioxid produced and the actual amount of carbohydrate material lost from the grain, per 100 gm. of dry matter, in the process of respiration throughout the 16-day ripening period. The amount of carbohydrate material lost was calculated from the data showing the carbon dioxid evolved. The evolution and estimation of carbon dioxid is probably the most accurate method of determining the loss in weight due to oxidation, and is intended to account for the entire collective activities of respiration. While the process of respiration is a complex one, and it is impossible to account for the exact changes which take place, the carbohydrate material lost can be very closely estimated by assuming the process in general to be oxidation of sugars and starch.

The oxidation process results in the formation of carbon dioxid and water according to the empirical formula, $C_6H_{10}O_5 + O_{12} \rightarrow 6CO_2 + 5H_2O$. One part of carbohydrate with a molecular weight of 162 when oxidized by free oxygen, results in the formation of 6 molecules of carbon dioxid with an equivalent weight of 264. From these figures it will be seen that the formation of 100 gm. of carbon dioxid will require 61.36 gm. of carbohydrate material. Thus, the amount of carbohydrate lost is calculated as 61.36 per cent of the carbon dioxid evolved. For wheat this gives a loss of 10.038 gm.; oats, 11.092 gm.; and barley, 15.12 gm. of carbohydrate per 100 gm. of dry matter in 16 days, as indicated in Table I.

TABLE I.—*Carbon dioxid evolved, and carbohydrate material lost by respiration, from wheat, oats, and barley, through a 16-day ripening period*

Crop.	Date.	Weight taken.	Dry matter.	Moisture.	Weight of dry matter.	CO_2 respired in 2 hours.	CO_2 respired per hour, per 100 gm. dry matter.	CO_2 respired in 24 hours, per 100 gm. dry matter.	Carbohydrate lost in 24 hours, per 100 gm. dry matter.
	July.	*Gm.*	*Per cent.*	*Per cent.*	*Gm.*	*Mgm.*	*Mgm.*	*Gm.*	*Gm.*
Wheat	5	23.70	56.13	43.87	13.30	28.39	106.73	2.561	1.571
	6	24.14	54.27	45.73	13.10	31.30	119.47	2.867	1.759
	7	25.00	60.25	39.75	15.06	26.13	86.85	2.084	1,278
	8	25.00	60.00	40.00	15.00	21.48	71.60	1.718	1.054
	9	25.00	59.10	40.90	14.77	20.28	68.66	1.647	1.010
	11	25.00	66.80	33.20	16.70	18.41	55.12	1.322	.811
	12	25.00	76.66	23.34	19.16	16.26	44.26	1,062	.651
	13	25.00	81.78	18.22	20.44	6.46	15.81	.379	.232
	14	25.00	77.17	22.83	19.29	6.72	17.42	.418	.256
	15	25.00	78.74	21.26	19.68	7.85	19.94	.478	.293
	16	25.00	84.87	15.13	21.22	3.33	7.85	.188	.115
	18	25.00	80.27	19.73	20.07	3.88	9.67	.232	.142
	19	25.00	85.21	14.79	21.30	3.49	8.19	.196	.120
	20	25.00	85.56	14.44	21.39	4.94	11.55	.277	.170
	21	25.00	87.50	12.50	21.87	10.01	22.89	.549	.336
	22	25.00	87.29	12.71	21.82	7.11	16.29	.391	.340
								16.369	10.039
Oats	11	25.00	58.62	41.38	14.65	29.49	100.65	2.415	1.481
	12	25.00	51.75	48.25	12.94	35.63	137.67	3.304	2.027
	13	25.00	52.40	47.60	13.10	24.87	94.92	2.278	1.397
	14	25.00	64.91	35.09	16.23	21.01	64.73	1.553	.953
	15	25.00	65.36	34.64	16.34	19.54	59.79	1.434	.880
	16	25.00	71.06	28.94	17.76	17.12	48.20	1.156	.709
	18	25.00	77.25	22.75	19.31	12.11	31.36	.752	.461
	19	25.00	79.79	20.21	19.95	11.30	28.35	.680	.417
	20	25.00	83.38	17.62	20.59	6.46	15.69	.376	.231
	21	25.00	79.25	20.75	19.81	9.69	24.58	.590	.362
	22	25.00	85.41	14.59	21.35	8.40	19.68	.472	.289
	23	25.00	83.88	16.12	20.97	13.24	31.57	.757	.464
	25	25.00	86.09	13.91	21.52	17.45	40.54	.973	.597
	26	25.00	89.60	10.40	22.40	7.75	17.30	.415	.254
	27	25.00	90.09	19.91	22.52	7.92	17.58	.422	.258
	28	25.00	85.42	14.58	21.35	5.98	14.00	.336	.206
	29	25.00	84.99	15.01	21.22	3.07	7.23	.173	.106
								18.086	11.092
Barley	5	16.983	47.99	53.01	8.13	48.61	298.95	7.175	4.402
	6	16.619	47.79	52.21	7.94	40.37	254.22	6.100	3.742
	7	25.00	53.98	46.02	13.49	23.26	86.21	2.070	1.270
	8	25.00	58.24	41.76	14.56	25.19	86.50	2.080	1.276
	9	25.00	56.13	43.87	14.03	19.35	69.07	1.660	1.018
	11	25.00	68.65	31.35	17.16	16.63	48.75	1.170	.717
	12	25.00	68.62	31.38	17.16	16.67	48.57	1.165	.714
	13	25.00	71.57	28.43	17.89	9.21	25.74	.617	.378
	14	25.00	75.53	24.47	18.88	6.80	18.01	.432	.265
	15	25.00	81.82	18.18	20.45	3.07	7.51	.180	.110
	16	25.00	86.22	13.78	21.55	2.68	6.22	.149	.091
	18	25.00	85.29	14.71	21.32	2.91	6.59	.158	.097
	19	25.00	86.52	13.48	21.63	7.75	17.91	.429	.263
	20	25.00	86.04	13.96	21.51	4.94	11.48	.275	.168
	21	25.00	87.85	12.15	21.96	9.37	21.32	.512	.314
	22	25.00	86.71	13.29	21.68	8.40	19.38	.465	.285
								24.637	15.120

It was the intention to show the relative variation between wheat, a naked grain, and oats and barley, to which the glumes adhere, in the amount of material lost. As oats and barley are apparently less variable in their protein percentage than wheat,[4] it was thought that the adhering glumes might be influential in inhibiting respiration and consequently account for this lesser fluctuation. This demonstration was made impossible through inability to obtain samples of uniform moisture content, which, no doubt, is largely responsible for the wide difference in the results.

EFFECT OF RESPIRATION UPON PROTEIN PERCENTAGES

The result of the loss of carbohydrate material as affecting a variation in the protein percentage can be ascertained by estimating the percentage of the grain which is subjected to respiration activities. The composition of the average grain is approximately 12.50 per cent protein, 2.50 per cent ash, and 85 per cent carbohydrate. Respiration being confined to the carbohydrate portion of the grain, it would require approximately 7 per cent variation in this material to effect a 1 per cent difference in the protein percentage. As the number of grams of carbohydrate loss, in this case, is based upon the 100 gm. dry basis, these same figures represent the loss in percentage. The protein will vary 1 per cent for each 7 per cent loss or gain in carbohydrate.

Calculating from the amount of carbohydrate material lost as given in Table I, it becomes apparent that there is a possible variation in wheat of 1.46 per cent, oats 1.62 per cent, and barley 2.2 per cent protein, due to respiration.

While these figures seem upon first thought to be definitely significant, it is not probable that respiration creates a variation in the protein content as wide as the above possibilities suggest. As indicated, considerable carbohydrate is lost during the ripening period, but fresh material is no doubt being moved into the grain until complete maturity, which is sufficient at least to counterbalance that which is lost. It must be recognized, however, that the amount of material being moved into the grain will be a variable factor and dependent largely upon the environmental conditions which prevail at that time. Brenchley (*1*) has shown that in the development of the barley grain the maximum dry weight of 1,000 gm. is reached when the moisture content is approximately 40 per cent, and that this dry weight remains practically constant and does not fall off during the desiccation process, while the moisture content is being lowered to 21 per cent. The fact that the weight of dry matter is not lowered indicates that up to the time of maturity considerable material is being moved into the grain to compensate for that lost by respiration.

More confirmation of this supposition is presented by Harlan (*3*) in his study of the development of the barley grain, where he has shown that deposits of dry matter in the kernel continue until very near the point of absolute ripeness. He concludes (*4*) from irrigation studies that plants are able to utilize water to the date of full maturity, with much increase in dry matter content.

[4] McGINNESS, F. W. OP. CIT.

The fact that the movement into the grain is continuous while respiration activities are in operation makes it impossible to determine the exact effect of respiration upon the variation in protein percentage. Assuming that the possible variation in protein is as great as mentioned above, namely, 1.46 per cent in wheat, 1.62 per cent in oats, and 2.20 per cent in barley, this would not be sufficient to account for the wide variation in the percentage as found in grain when produced in different localities, as given in Table II.

Upon analysis of Table II, a very wide variation in protein percentage is found for the grains grown in different localities and also for different years when grown in the same locality. In the year 1921 wheat grown in the hard spring wheat section at Fargo, N. Dak., yielded 16.10 per cent protein, while the same variety at Puyallup, Wash., shows 9.98 per cent. Another extreme variation may be seen by examination of the results at Havre, Mont., showing a protein content of 18.24 per cent, and Waterville, Wash., 9.19 per cent in the year 1920. In the former case there is a variation of 6.12 per cent in this regard, and 9.05 per cent in the latter. It would not be possible to attribute these differences to the respiratory process alone, as the above results show a 1.46 per cent variation in the protein which can be due to this phenomenon.

The data for oats indicate a much greater uniformity in this respect than is found in wheat. While this uniformity is especially pronounced throughout the year 1920 for the different areas, there are several cases in 1921 which vary considerably. The oats produced at Moccasin, Mont., Moro, Oreg., and Umatilla, Oreg., have a protein content which will average 14.12 per cent. Those grown at Waterville and Puyallup, Wash., and Corvallis, Oreg., average approximately 11.32 per cent. The spread in oats is not so great as in wheat, but this fluctuation of 2.80 per cent is much greater than that caused by the effect of respiration.

While the loss of carbohydrate material from the barley grain is much greater than that from wheat or oats, owing probably to the higher moisture content, it is not sufficient to account for the protein variation where the grain is grown under different conditions. Barley grown at Fargo, N. Dak., in 1920, yields 16.06 per cent protein, while that produced at Corvallis, Oreg., yields only 11.11 per cent. This is a difference of 4.95 per cent, which is more than twice the difference which could be due to respiration. In 1920, 14.50 per cent barley was produced at Havre, Mont., and Logan, Utah, yields 11.11 per cent. Here, again, is a spread of 3.39 per cent in the protein content, or 54 per cent greater than that attributed to respiration.

In a number of the analyses of each of the grains, the differences in protein percentage falls within the respiration possibility. These smaller differences may or may not be due to respiration, depending largely upon the environmental conditions under which the grain is matured. It is quite possible that respiration is effective to a degree in shaping the percentage composition of the grains when grown in the areas as indicated in Table II, but it is also possible that respiration plays a rather unimportant rôle in this regard when the synthetic process which goes forward throughout the period of desiccation is taken into account.

The exact influence of respiration upon the protein percentage composition of the grain is yet to be determined, but there is no foundation for the general sweeping statement that the variation in composition is dependent upon the respiratory process.

TABLE II.—*Physical and chemical composition of wheat, oats, and barley grown in 16 different localities in 1920–21*

Crop and locality.	Weight 1,000 grains.		Corneous.		Protein.	
	1920	1921	1920	1921	1920	1921
Wheat:	*Gm.*	*Gm.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Original seed	34.20	34.90	38	80	12.80	12.57
Havre, Mont	19.60	29.75	100.	100	18.24	18.91
Moccasin, Mont.	25.85	25.00	100	95	15.85	16.00
Fargo, N. Dak.	28.90	22.50	100	100	12.34	16.10
Morris, Minn.	18.25	24.05	100	100	14.68	15.01
St. Paul, Minn	24.00	25.80	92	91	13.42	16.46
Duluth, Minn.	28.75	21.50	67	100	13.80	11.76
Grand Rapids, Minn	27.80	17.80	100	100	12.43	17.79
North Platte, Nebr.	20.00	22.70	100	100	14.55	15.01
Lincoln, Nebr.	25.00	20.60	100	94	12.81	14.52
Garden City, Kans.	22.10	19.30	98	100	15.24	15.10
Moro, Oreg	26.10	23.95	100	100	16.82	15.88
Umatilla, Oreg	24.25	18.60	100	100	15.57	18.52
Waterville, Wash.	30.35	33.85	0	98	17.10	15.65
Puyallup, Wash.	38.80	38.55	22.5	23.0	9.19	9.98
Corvallis, Oreg.	33.08	34.17	75.0	81.0	12.40	10.47
Logan, Utah	30.24	34.60	100	100	14.22	14.97
Barley:						
Original seed	34.45	36.70	.....	.....	12.46	12.48
Havre, Mont	19.00	31.35	.....	.....	14.50	11.21
Moccasin, Mont.	24.65	33.10	.....	.....	14.01	13.81
Fargo, N. Dak.	36.05	22.22	.....	.....	12.66	16.06
Morris, Minn.	25.10	33.70	.....	.....	11.08	13.13
St. Paul, Minn	28.22	24.70	.....	.....	11.06	13.01
Duluth, Minn.	32.16	40.15	.....	.....	14.00	11.08
Grand Rapids, Minn.	33.75	27.15	.....	.....	11.92	12.42
North Platte, Nebr.	25.75	28.15	.....	.....	12.44	13.03
Lincoln, Nebr.	24.15	31.05	.....	.....	11.83	12.33
Garden City, Kans.	22.12	23.52	.....	.....	12.12	12.41
Moro, Oreg.	17.50	28.40	.....	.....	13.05	10.88
Umatilla, Oreg.	27.79	16.75	.....	.....	12.62	13.71
Waterville, Wash.	36.11	36.00	.....	.....	14.34	13.89
Puyallup, Wash.	33.75	37.45	.....	.....	10.70	11.88
Corvallis, Oreg.	33.25	38.75	.....	.....	11.14	11.11
Logan, Utah	37.60	33.45	.....	.....	11.11	12.11
			Hull.			
Oats:						
Original seed	26.20	29.90	29.70	27.32	11.90	11.77
Havre, Mont	24.60	28.55	25.22	28.63	13.82	13.29
Moccasin, Mont.	26.45	23.25	25.00	25.76	13.67	14.20
Fargo, N. Dak.	30.36	30.00	23.12	26.12	11.11	12.40
Morris, Minn.	27.75	28.55	26.12	27.30	13.47	11.39
St. Paul, Minn	25.40	21.45	26.70	32.29	11.96	12.23
Duluth, Minn.	30.30	22.70	28.74	28.20	11.35	12.22
Grand Rapids, Minn	30.13	28.70	24.87	30.01	12.52	12.10
North Platte, Nebr.	27.05	19.00	28.03	22.00	12.25	12.26
Lincoln, Nebr.	27.70	21.00	25.56	21.00	11.78	13.94
Garden City, Kans.	28.80	21.40	28.40	24.60	11.36	12.15
Moro, Oreg.	24.65	27.30	29.36	30.02	13.69	14.05
Umatilla, Oreg.	26.65	28.10	34.21	25.90	12.21	14.23
Waterville, Wash.	27.60	31.80	27.64	30.66	12.25	11.31
Puyallup, Wash.	32.10	33.20	25.78	26.21	11.10	11.20
Corvallis, Oreg.	29.80	33.50	27.04	30.12	11.22	11.45
Logan, Utah	28.95	33.05	27.48	23.82	11.39	12.04

SUMMARY

(1) The loss of carbohydrate material during the ripening of the wheat, oats, and barley grains is considerable. The greater percentage of the loss occuring before the process of desiccation begins while the grain contains above 40 per cent moisture.

(2) The protein composition of wheat, oats, and barley is influenced to a marked degree by the loss of carbohydrate material during the ripening period. Factors other than respiration or in connection with the process contribute largely to the formation of high protein grains.

(3) Wheat is more variable in the protein percentage than barley or oats, and barley appears to be more variable than oats.

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